

Making new industries from old?

Mrs Margaret Thatcher's conference next week on wealth creation is badly named. Here is a guide for non-government speakers. But the government's tone of voice will be more important.

GOVERNMENTS of all kinds believe that research is one of the high roads to prosperity, but most of them behave as if they have been cheated of the benefits that should be theirs. This is the spirit in which governments of developing countries continually complain that "technology transfer" is inadequate and inequitable and in which governments of industrialized states are forever devising new schemes for increasing the economic yield from what happens in laboratories. Thus the United States Administration keeps brooding xenophobically about the economic losses which its taxpayers supposedly suffer from too free a flow of technological information and is busily encouraging schemes by which corporations can collaborate on strategic research within the framework of the anti-trust legislation.

Introspection

Britain, which has a longer history than most of introspection in this field, is also going through a period of renewed institutional (if not technological) innovation under the new slogan "wealth creation" in which the most remarkable development so far is the conference called for next Monday by the Prime Minister, Mrs Margaret Thatcher, who is well known not merely as the first woman to hold her present job but as the first Prime Minister with a science degree. The plan is that 200 people will be jawed at for a day by a panel of government ministers, industrialists, bankers, committee chairmen and one academic. If the participants are given a chance to answer back, this is what they should say under the various headings advertised in advance.

Wealth creation is an activity at which Britain was skilled about a century and a half ago, but at which it has been steadily eclipsed since the 1870s. Three sets of circumstances account for this apparently inexorable industrial decline — the structure of successful British companies (which appear still to model themselves on hierarchical institutions such as the eighteenth century's East India Company), the British inclination over several decades to put social civility before prosperity (whence high public expenditure, sharply "progressive" taxes, trades union rights, a low savings ratio and a general sense that Britain is a congenial place in which to live) and then, more recently, government intervention in the support of backward industries (whose failure would be politically embarrassing) or hare-brained technological schemes (such as the Concorde aircraft). Even so, wealth creation has not been negligible. Over the years, the British gross domestic product has steadily increased. What irks the government and those whose taxes it spends is that the rate of growth has for so long been so much less than that of other industrialized countries that Britain is no longer particularly prosperous. This outcome was of course predicted by the Barlow committee in its recommendations on technical manpower in the 1940s, by government-sponsored organizations such as the Anglo-American Productivity Council in the 1950s and by a host of later commentators.

Innovation. It is fashionable to say (as the Prime Minister herself is fond of doing) that Britain is first rate at the generation of new ideas but poor at their exploitation. The most frequently-cited illustration is still the British discovery of the first antibiotic in the 1930s. The British failure to exploit the early development of fully electronic computers with substantial built-in storage

(1947) or to found a substantial civil aviation industry on the early application of jet engines (mid-1950s), economically much more important, is the responsibility of those who failed to appreciate the scale on which money and manpower must be devoted to such enterprises. The history of more recent decades is littered with other projects (coal gasification, direct reduction of iron oxide to steel, computer-controlled machine tools and even the British Broadcasting Corporation's early but wrong-headed development of a video-recording system) that have failed because those responsible have substituted optimism for a knowledge of the market at which they were aiming and have consistently supposed that they could safely skimp on resources in the expectation that native-born flair would somehow make good deficiencies elsewhere. Thus those who insist next week on referring to wasted opportunities should be asked also to acknowledge that:

- Major industrial innovations depend more on resources and market appraisal than on flair, and will succeed only if their outcome is qualitatively superlative. To be second or third best is to fail (witness British Telecom's digital switching system known as "System X").
- Smaller projects, those within the scope of a single small or medium-sized company, similarly require attention to the market and to quality. The British record is not nearly as bad as is commonly supposed (cephalosporin antibiotics and many other pharmaceuticals for example), while there is no long list of ideas whose exploitation has been muffed. On the contrary, the legend that Britain is a state overflowing with industrial innovations that others are about to steal is a conceit.
- Most technological innovation stems not from original "ideas" but consists of the improvement of products and processes that already exist. To be best at doing something is, in the present international climate, all that matters. Japanese companies have shown what rewards success can bring. Success, however, requires engineers who are not merely able but also zealous and companies that appreciate their importance. The familiar scorn of many British companies for the engineers they employ (why else would they pay them so badly?) explains a lot.

Generalities

With luck, next week's conference will accept such generalities (although the notion that Britain is no longer, if it ever was, super-creative will stick in many throats). Difficulties arise about the steps that should now be taken. Unlike its predecessors, the present British Government will be disposed to preach next Monday on the familiar text that a government cannot practise industrial innovation but only create a climate in which innovation has a chance to prosper. (Amen! should be the response.) It has, however, given a hostage to fortune by offering several of its ministers as cheerleaders for Mrs Thatcher's conference. Here are some questions they should be compelled not to dodge.

Defence research has since the Second World War consumed a half of all spending on research and development and a greater proportion of direct government expenditure. Technology transfer between the defence research establishments and the rest of British industry has always been inadequate and will remain so while the armed services insist on self-sufficiency in research in those areas in which they can still afford to develop new weapons of their own. Mr Michael Heseltine, the Minister of Defence,



should therefore be asked next Monday when defence research, at present uncontrolled, will be put within the purview of government committees surveying public support for research and development. If he is serious about wealth creation, he should also agree that future defence research and development projects will be restricted to those calculated to yield by-products in civil industry, and that other weapons will be bought from suppliers elsewhere. If Mr Heseltine says that the result would be a loss of jobs in defence manufacturing industries, he should be reminded that it makes no sense that half of Britain's research and development spending should be used to sustain the defence procurement business, even with export sales no more than 5 per cent of gross domestic product.

Information technology may be mistaken for a personal creation of Mr Kenneth Baker, whose ministerial portfolio at the Department of Trade and Industry has that title. Mr Baker will no doubt rehearse again next week his now-familiar message that there are splendid opportunities for British industry (as for other people's industries) in the nexus between telecommunications and the manufacture and application of computer technology, and will mention what the government has done so far to make this field of technology fashionable (with special new posts at universities, the £400 million collaborative programme with industry on computer design and technology and the encouragement of cable television). Mr Baker should be asked, however, to explain what exactly he means by the title of his office, how exactly his optimism has survived disappointments (to make no more of them) such as British Telecom's "System X" and how he and his colleagues expect that further innovations will prosper.

Education will be dealt with lopsidedly next week by Sir Keith Joseph, who is responsible only for the formal educational system in Britain and for the funds the research councils spend on civil science. (Other training schemes, mostly inadequate, fall between the Departments of Trade and Industry and of Employment.) Many practical questions (such as the size of next year's science budget) will easily be dodged on the grounds that they have not yet been decided. But the minister should be asked how he can defend the present circumstances in which British universities are compelled by arbitrary student quotas to be less efficient than they should be, and to live with a balance between arts and science students dictated from on high, even though the national need may coincide with students' needs for more technical education. The minister should also declare openly where he stands on the now-fashionable folly of believing that universities must contribute to wealth creation not merely by making young people skilled at it but also by carrying out research likely to make a direct contribution to the same cause (which may often have the opposite effect). Hitherto, the British Government has been so insensitive on this issue that it has not seen that an issue exists.

Trade and Industry is now one of the most powerful departments in the British administration, partly because its new minister, Mr Cecil Parkinson, is also chairman of the Conservative Party. The department has an unenviable record, having been in its time the chief source of previous governments' conviction that all academic institutions are a waste of money, an unsuccessful punter in high technology and one of the chief superintendents of the nationalized industries (whose research programmes have been shamefully neglected). Mr Parkinson, still new, should without prompting next week declare himself against helping to support industries whose economic future will be shaky even if economic circumstances improve (shipbuilding, steel), not only for the sake of saving public money but so as to make room in the economy for the new industries on which the government has set its heart. Mr Baker's enthusiasm should be restrained or, better, made discriminating. The government's support for small business (in Mr Parkinson's full charge) should be less trendily geared towards entirely new companies, while government schemes for post-school training should, for the sake of young people as well as wealth creation, be concerned more explicitly with the skills the future needs. The nascent interest of Mr Parkinson's department in formal education, perhaps as a partner in supporting some universities, is to be welcomed, but the time is

fast approaching when somebody should say what it amounts to; next Monday would be a good opportunity.

Delicacy

Mrs Thatcher's task next week will be more delicate for, like other conference organizers, she will have to make up for the deficiencies in the programme she has provided. No doubt the venture capitalists due to speak will confirm the general impression that British investors are now more willing to take risks with money, and there is not yet enough unhappy experience on the newish unlisted securities market to shatter this new-found faith in high-tech entrepreneurial companies. But the Chancellor of the Exchequer, Mr Nigel Lawson, will not be present to offer his own solution to the long-standing British dilemma thrust on him, like his predecessors, by slow growth — the choice between cutting public spending (less training, less direct support for innovation?) and putting up taxes (discouraging entrepreneurs and their backers). Yet these are crucial determinants of the climate-creation business on which the government will be talking next week. Can Mrs Thatcher promise that this time the government will cut spending that is irrelevant to its professed cause of wealth creation (much defence research, support for antiquated industries, for example) whatever the political costs?

She also has a more subtle task, to give an acceptable account of what is meant by the unfortunate slogan wealth creation. Her first government was correct in supposing, four years ago, that Britain had drifted through many years of unreasonable expectations into a state of affairs in which industrial innovation had become either unprofitable or impracticable. Much of its past policy has been aimed at striking a different balance between people's sense of personal security in all economic circumstances and the sense that those who contribute to industrial innovation, or those who work for them, should be equitably rewarded. The defect of the slogan with which next week's conference is lumbered is that it suggests that wealth is a sufficient objective in itself. To the extent that more gross domestic product may in the long run mean that more people have interesting jobs to do, or that it is possible to meet acknowledged social objectives while keeping taxes within reason, wealth may be a valuable indicator of success in meeting other objectives. The snag, in the past four years, is that too many spokesmen for the British Government have talked to a much narrower definition. Many of those most often regarded as crucial to the government's crusade, academic scientists in particular, have no reason willingly to trade their present interests for a set of objectives wished on them by a government for whose reelection they may not have voted. Mrs Thatcher's task is thus to persuade her audience (or those in laboratories to whom her audience will be looking for creativity) that her crusade is not partisan.

Demeanour

Close attention will therefore be paid to the terms in which this is done. Like other occasions of the kind, next week's conference is an exercise in public relations which is not to be scorned on that account. Mrs Thatcher's previous government was right in its conclusion that the scale of government expenditure, and the detailed character of government intervention in industrial innovation, had become respectively impoverishing and counter-productive. Many of the steps taken in the past four years have been sensible. Others have been disastrous mistakes (such as the manner in which university budgets have been cut). If the second Thatcher government can use next week's conference as a chance clearly to explain why it has made productive industrial innovation a central part of its programme, if it can show how its day-by-day policies can contribute to that end and if it can persuade those not so prejudiced that they cannot listen that the objective is not to make millionaires out of a few PhDs but to generate resources that might be used (even by a government of quite different stripe) for much wider purposes (such as the preservation of the peculiarly British civility), the result could be electrifying. The technical community, the putative origin of all this wealth, needs some encouragement. But everything will depend on the tone of voice. □

Scandinavian acid rain

Royal Society appointed referee in UK dispute

THE Royal Society of London, in conjunction with the Norwegian Academy of Science and Letters and the Royal Swedish Academy, has embarked on a five-year study of the contentious problem of acid rain in northern Europe. The development breaks new ground for the society, whose formal studies of public issues have hitherto been confined to uncontroversial topics, those on which its advice has been sought by government or which appeared to lie within the professional interests of its members.

The new study is also unprecedented in that it will be supported to the tune of £5 million over five years by the two British nationalized industries most likely to be adversely affected by a finding that sulphur dioxide emissions from British power stations are responsible for the disappearance of fish from lakes in Scandinavia. Both the Central Electricity Generating Board and the National Coal Board have agreed to provide up to £2.5 million each for the study. Its director will be Sir John Mason, who retires as director-general of the Meteorological Office at the end of this month.

The origin of the study appears to rest with Sir Walter Marshall, a fellow of the Royal Society since 1971 and now chairman of the Central Electricity Generating Board. At the announcement of the study earlier this week, he explained that he could not on the basis of the evidence now available agree that emissions from the board's chimneys should be freed from sulphur dioxide at a cost of £4,000 million, adding some 10-15 per cent to the cost of generating electricity. Mr Ian MacGregor, the new chairman of the National Coal Board, took the same line earlier this week.

Everybody is anxious to emphasize that the study of acid rain and its effects will be carried out independently both of the sponsors and of the governments concerned. The participation of the Norwegian and Swedish academies is, however, a guarantor of that independence. The planning of the study will be supervised by a management group under Sir Maurice Sugden and will consist of four nominees of the Royal Society and four nominated by the Scandinavian academies (not yet chosen). The announcement says that the results of the new research programme will be published "without restrictions".

The British membership of the management group has the additional interest of including Professor T. R. R. Southwood (Oxford), who is the present chairman of the Royal Commission on Environmental Pollution, supported financially by the

government. The other members are Dr T. F. West, Professor E. J. Denton and Dr J. F. Palling (from the Freshwater Biological Association).

The plan is that the study should not be concerned with the airborne transport of sulphur dioxide from one place to another, on the grounds that enough work on that subject is already under way, but that it should concentrate instead on the effects of acid rain on land and water. The effects of airborne sulphur dioxide on forest species may also be left aside on the grounds that these are being taken up energetically in West Germany and elsewhere.

In their announcement of the new study, the three academies give as an example of the problems to be investigated the recognition within the past decade that the death of fish in Scandinavian lakes may not be the direct result of acidity as such but rather of the mobilization of aluminium from surrounding catchment minerals.

The Central Electricity Generating

Board, on its own behalf and that of the National Coal Board, refers without dissent to the study by the Organization for Economic Cooperation and Development (OECD) in 1976 which suggested that Britain might be responsible for half the acid deposition in Norway and Sweden.

While guessing that its own chimneys may be responsible for half the sulphur dioxide put into the atmosphere in Britain, the generating board also insists that there is at present no way of calculating how much benefit would result in Scandinavia from a specified reduction of emission from the United Kingdom. The boards also raise the interesting suggestion that the sulphur dioxide concentration of the atmosphere may predate the rapid growth of electricity generating plant in this century.

By their approval of the United Nations convention on long-range transport of air pollutants and its advocacy of research on such problems, the two British boards also implicitly accept responsibility for doing what they can to mitigate such problems as can be laid at their door. By commissioning the Royal Society to carry out the study, they have for practical purposes appointed it the referee in deciding what should be done to abate the nuisance of acid rain. It will be interesting to see how well the society discharges that role five years from now. □

Dalai Lama visits nuclear physicists

It seems that 1983 is rapidly becoming a religious year for CERN, the European Organization for Nuclear Research near Geneva, where the intermediate vector bosons (W s and Z^0) were discovered. A few months ago His Holiness the Pope passed by and gave his blessing; last month it was the turn of the Dalai Lama, spiritual leader of the Tibetan Buddhists.

The Dalai Lama and his retinue had apparently asked to be briefed on the objec-

ted in the vacuum, Jacob said, because vacuity is an important concept within Buddhism. No object exists to a Buddhist unless it is recognized and distinguished — the element of consciousness must enter. But, Jacob explained, there is a big difference between vacuity — complete nothingness — and the physical vacuum, which is obtained when all particles and fields are removed to produce a state of lowest energy. Such a vacuum "has all of physics in it", as it could be polarized — particles and antiparticles created from it — by a probe. What this has to do with consciousness is unclear, but the Dalai Lama went so far as to suggest that science could aid the interpretation of scripture.

The Pope, on the other hand, had been less interested in the philosophy of science and more in its social impact — as befits the relative natures of Christianity and Buddhism. He asked CERN researchers to be vigilant about nuclear war, and to use their expertise to help alert the public to the dangers of nuclear conflict.

Both religious leaders stressed a continuity between science and faith. The more hopeful ecumenists who favour a *rapprochement* of all the higher religions may take this as a good sign, for if A is continuous with B, and B with C, then so is A with C, provided B is continuous. Since most scientists believe in the logical — or at least existential — continuity of science, this implies that Christianity is continuous with Buddhism.

Robert Walgate



tivity of science, on determinism and quantum mechanics, and on the nature of the vacuum. They were fed this stimulating but somewhat indigestible fare over lunch by the CERN ex-director Leon Van Hove (objectivity), John Bell (determinism) and Maurice Jacob (vacuum).

According to Jacob, the Dalai Lama "had a lot of sharp questions". He was in-

Hazardous wastes

Give US laboratories a break

Washington

WASTE-disposal procedures in US laboratories should be greatly simplified, according to a National Academy of Sciences study that has just been published. The recommendations are a response to the frustrations felt by researchers in trying to comply with the present complicated federal regulations governing the disposal of hazardous waste.

The regulations which have been in effect since 1980 were designed to halt the haphazard and back-door methods of disposing of hazardous substances that came to public attention through such notorious cases as the "Valley of the Drums", where thousands of corroding 55-gallon steel drums had been dumped. The problem, says Dr Robert Alberty of Massachusetts Institute of Technology, the chairman of the academy study, is that the regulations were designed primarily with industry, not laboratories in mind. Speaking at last week's American Chemical Society meeting, Alberty said that the rules place a burden on laboratories "out of proportion to the fraction of hazardous waste that laboratories generate and to the overall hazard it poses".

According to estimates by the Environmental Protection Agency (EPA), laboratories account for only 0.1–1 per cent of the waste defined as "hazardous" under the present regulations. These include toxic, corrosive, combustible and reactive chemicals. Radioactive wastes are covered by a different set of rules.

The hazardous waste regulations require that each producer of waste keep extensive records of each specific hazardous chemical generated and that a system of shipping manifests be used to ensure that waste is delivered properly to an approved landfill or disposal facility. Laboratories tend to produce small quantities of a great many different substances, which can result in a particularly onerous record-keeping burden. Even a collection of used filter paper, residues of chromatographic separations and minor by-products from small-scale tests would have to be listed, by specific chemical content, on a shipping manifest for disposal. "No useful purpose is served by listing what may be more than 100 individual small samples" the academy study says.

Instead, the study recommends that wastes from laboratories be labelled, and records kept, only in terms of seven broad categories: reactive, toxic, ignitable, acidic, basic, oxidizers and a miscellaneous category for the filter-paper and crusty-test-tube sort of trace quantity waste. A drum containing laboratory wastes so identified could be shipped to a disposal site, the study proposes, so long as no containers in the drum were larger than 20 litres.

The study also criticizes the existing

regulations for effectively forcing laboratories to use landfills rather than incineration for disposing of waste. Although incineration is potentially safer and more convenient than landfill disposal, commercial incinerators usually refuse to accept

Radioactive waste

UK ocean dumping in dispute

GREENPEACE, the "direct action" environmentalist organization, has stepped up its campaign to embarrass the British Government over dumping of low-level radioactive waste in the sea. Greenpeace has released copies of a restricted government document which, it is claimed, indicates that the Ministry of Defence (MoD) is secretly planning to include in the scheduled 1984 dump in the Atlantic plutonium-containing wastes from the Atomic Weapons Research Establishment at Aldermaston.

Friends of the Earth, the more sedentary but equally vocal UK environmental group which has been campaigning for more freedom of information, congratulated the unnamed public servant who leaked the document for bringing the affair into the public eye. The document consists of minutes of a meeting between representatives of MoD and the Ministry of Agriculture, Fisheries and Food. Although the

laboratory waste because of the small quantities and great chemical diversity. The study says that installation of small incinerators at laboratory sites could be encouraged by waiving the expensive requirement for a test burn before a licence can be granted. The academy study recommends that EPA should adopt a conservative design standard as a substitute for the test burn requirement. **Stephen Budiansky**

minutes do not describe MoD's proposal in detail, it appears that the plan was to dump waste containing between 200 and 600 grams of plutonium in 4 or 5 containers. Much of the discussion at the meeting centred on whether the proposed containers, which are larger than the type used for most low-level waste, would "attract attention" and "lead to awkward questions about the contents and origin of the packages".

MoD says that no decision has yet been taken on the plan, but that the waste would certainly come within the International Atomic Energy Authority's (IAEA's) definition of low-level waste suitable for sea dumping. For alpha-emitters, the limit is 1 curie per ton averaged over 1,000 tons. Independent authorities confirm that, with suitable dilution and making certain assumptions about the isotopic composition, this quantity of plutonium could easily be packaged so as to come within the IAEA definition. The activity of the waste is estimated to be in the region of 30 curies, which is less than 3 per cent of the alpha-curies dumped by Britain in 1982. Greenpeace's claim that the proposal would "defy all international regulations" therefore seems difficult to sustain.

Last February, the London Dumping Convention adopted by 19 votes to 6 (with 5 abstentions) a resolution calling for all sea dumping of radioactive materials to be suspended pending an expert report which will be available for the convention's 1985 meeting. The resolution, which is not legally binding, stemmed from a proposal from two Pacific nations, Kiribati and Nauru; the countries that voted against were Britain, the United States, Japan, the Netherlands, South Africa and Switzerland. After the convention meeting, Britain announced that it would proceed with plans to dump 4,000 tons of low-level waste this summer. Britain accounts for about 80 per cent of all nuclear waste dumped at sea. In the event, the British plans appear to have been thwarted by industrial action by the National Union of Seamen, supported by several other transport unions. The union's executive instructed its members to black the cargo, and the dumping vessel, *Atlantic Fisher*, has not yet sailed. NIREX, the nuclear waste disposal executive, is now looking for suitable land dumping sites.

Tim Beardsley

Medal invented

Washington

THOSE who accuse President Reagan of favouring form over substance were recently handed some new ammunition. Having apparently decided that its plan to reorganize the Department of Commerce was not enough to stimulate technological productivity (even if the new name — the Department of International Trade and Industry — would make everyone think of Japan's awe-inspiring Ministry of International Trade and Industry), the administration has now come up with a new presidential medal.

"The President is eager to recognize innovators in technology who have helped America compete successfully in the international market place", said George Keyworth, the President's science adviser, in announcing the new National Medal of Technology. Nominations will be accepted by the Department of Commerce until November 30*. To help nominators, the White House offered this pointer: "Had the medal existed in the past, it might have gone to Thomas Edison for initiating urban electrification."

Stephen Budiansky
*Instructions and nomination forms are available from: Assistant Secretary for Productivity, Technology, and Innovation, US Department of Commerce, Washington, DC 20230.

Petroleum congress

London gathering optimistic

THE Eleventh World Petroleum Congress, which took place in London last week, marked the fiftieth anniversary of the first such meeting, also in London. This round date inevitably gave a certain historical perspective to an otherwise routine forum for the exchange of technical expertise. The mood, however, was forward rather than backward looking, although the Polish delegation took the opportunity to host a slightly belated commemoration of the centenary, last year, of the death of Ignacy Lukasiewicz, whose invention in 1853 of the kerosene lamp effectively launched the modern petrochemical industry. Where more modern history impinged on the congress, the results were less happy — the Argentinian delegation arrived without its technical handouts, which had apparently been impounded by the British customs authorities.

The general theme of the congress, petroleum in the twenty-first century, both reflected the "historic" aspect of the meeting and proved sufficiently broad to cover all practical aspects of the industry

from prospecting to refinery techniques and from hydrocarbon chemistry to rig safety. Although no definitive answer emerged to the vital question of just how large are the world's remaining petroleum reserves, a general consensus emerged that, as far as oil and gas are concerned, the situation is "more optimistic than pessimistic". Since, however, new oilfields can be expected in more and more remote and difficult areas, there will be increasing scope for international cooperation and the pooling of expertise. In some cases, such as the recent proposals for joint Norwegian-Soviet exploration of the Barents Sea, there could be difficulties over lines of demarcation, but so far the atmosphere remains amicable, and the congress heard many accounts of effective and mutually beneficial joint training schemes and transfers of know-how, such as the joint British-Chinese project, which involved on the one hand the training of Chinese technicians aboard North Sea rigs, and on the other a considerable British involvement in the exploration of the South

China Sea.

Cautious optimism about world petroleum reserves does not, however, lessen the importance of energy conservation and more efficient recovery and processing methods. Topics discussed at the congress included new methods of obtaining hydrogen from residues, *in situ* combustion, the recovery of hydrocarbons from oil shales and the liquefaction and gasification of coal.

The formal programme had, however, little to offer in answer to the Prince of Wales, who, in opening the congress suggested that oil entrepreneurs with "imagination and far-sightedness" would doubtless set aside funds to develop new forms of energy to replace oil in the next century. Nevertheless, at least one oil producer is about to diversify — a special number of the Yugoslav petroleum journal *Nafta* published for the conference reported that the Croatian petroleum production association INA is about to start pilot-plant production of uranium yellow cake from phosphoric acid.

Vera Rich

UK postgraduates

SERC cracks the whip

POSTGRADUATE students in British universities are taking too long to complete their PhD theses, according to a survey by the Science and Engineering Research Council (SERC). The survey asked the 59 universities and colleges in Britain how many of the students who began their PhD courses in 1978 had finished them within 4 years — by October last year. The table shows the results. Some students who are not still

registered after 4 years had left without presenting a thesis, hence the "missing" students in the table. SERC maintains that "writing up results within a reasonable timescale is an integral and important part of research training", and the council has circulated a booklet entitled *Research Student and Supervisor: a discussion document on good supervisory practice* in an attempt to improve matters. □

PhD submission by institution

Universities	No. of students registered	No. submitting by 1.10.82	No. still registered at 1.10.82 (%)	Universities	No. of students registered	No. submitting by 1.10.82	No. still registered at 1.10.82 (%)
Aston	62	26	21 (34)	Manchester	94	59	29 (31)
Bath	40	19	9 (23)	UMIST	55	20	9 (16)
Birmingham	67	42	18 (27)	Newcastle	56	24	30 (54)
Bradford	65	36	27 (42)	Nottingham	72	46	22 (31)
Bristol	65	36	27 (42)	Open	8	0	5 (63)
Brunel	15	7	7 (47)	Oxford	132	89	30 (23)
Cambridge	140	98	32 (23)	Reading	33	11	16 (48)
City	14	5	2 (14)	Salford	41	19	17 (42)
Cranfield Inst. of Tech.	10	3	4 (40)	Sheffield	69	32	31 (45)
Durham	20	10	9 (45)	Southampton	62	23	30 (48)
East Anglia	29	11	16 (55)	Surrey	32	19	11 (34)
Essex	18	4	9 (50)	Sussex	46	21	20 (43)
Exeter	22	10	12 (55)	Warwick	36	16	13 (36)
Hull	19	10	8 (42)	York	28	13	14 (50)
Keele	8	6	0 —	University of Wales:			
Kent	25	5	14 (56)	Aberystwyth	18	12	5 (28)
Lancaster	23	12	5 (22)	Bangor	24	8	8 (33)
Leeds	95	40	44 (46)	Cardiff	37	15	15 (41)
Leicester	38	19	12 (32)	Swansea	28	6	22 (79)
Liverpool	68	35	29 (43)	UWIST	10	4	4 (40)
London:				Aberdeen	21	11	7 (33)
Bedford	9	3	4 (44)	Dundee	13	9	3 (23)
Birkbeck	12	4	8 (67)	Edinburgh	44	20	21 (48)
Chelsea	16	7	8 (50)	Glasgow	45	22	12 (27)
Imperial	137	67	48 (35)	Heriot-Watt	24	10	11 (46)
Kings	24	14	6 (25)	St Andrews	21	16	4 (19)
Queen Elizabeth	21	3	16 (76)	Stirling	7	4	1 (14)
Queen Mary	37	11	25 (68)	Strathclyde	40	22	14 (35)
Royal Holloway	13	2	8 (62)	Queens Belfast	1	1	0 (—)
University	52	23	23 (50)	Total universities	2,297	1,124	868 (38)
Westfield	9	4	5 (56)	Total polytechnics	100	35	51 (51)
Other institutions	33	14	13 (39)	Other institutions	8	1	4 (50)
Loughborough	25	8	14 (56)	Total	2,405	1,160	923 (38)

Cleaner Thames

THE Thames Water Authority last week jubilantly announced that for the first time in 150 years a salmon had been caught in London's river. The authority began restocking the Thames with salmon in 1979 with the aim of seeing whether the river had been sufficiently freed of pollution to satisfy even the fastidious salmon. For catching the fish, Mr Russell Doig was rewarded with a prize of £250. What the salmon thought of today's River Thames is open to speculation, but the fish may not have been that impressed, as in Mr Doig's words "For a salmon it didn't put up much of a fight". □



British higher education

Changing pattern in polytechnics

THE upheaval in British higher education continues. Far-reaching plans for changes in the provision of advanced further education courses outside the universities were sent last week to more than 200 directors of polytechnics and other colleges supported by local authorities. The proposals, which are the result of the first major planning exercise by the National Advisory Body for Local Authority Higher Education (NAB), include for the first time provisional cash allocations derived from projected student numbers calculated on the basis of need rather than by tinkering with historic student numbers. Resources for science, engineering and computing courses will all be increased relative to other subject areas.

NAB's planning exercise has been conducted against a background of continuing reductions in the public provision for advanced further education. The provisional student target total for the academic year 1984-85 is a modest increase on the total for the coming year, but because of the high numbers of students now in the first and second years of their courses, between 5,000 and 10,000 new places will be lost compared with present enrolments. Expenditure per full-time student will nevertheless

be substantially reduced.

NAB has tried to balance student demand, geographical variations in the quality of existing provision and the need to increase support for specific kinds of studies. Target student numbers for individual institutions have not been made public, but it seems clear that their fortunes will vary widely. Most of the projected changes in cash allocations from the central pool in 1984-85 are based not on student targets resulting from the planning exercise itself but on a watered-down version arrived at by halving the changes predicated by that exercise. This peculiar "mitigation" procedure, which applies across the board, was a last-minute improvisation meant to soften the blow to institutions that would otherwise be left in a hopeless position. The "mitigation factor" is a remnant of a device used in previous years for the same purpose and will certainly be modified in discussions to be held with colleges and local authorities over the next few weeks in order to make it more selective. NAB's proposals have yet to be endorsed by its board and committee, while central government will not finally decide how much cash there is to spend until the autumn.

The mitigation factor apart, NAB's proposals mean that three or four colleges may have to close or to merge with other institutions — unless they can persuade NAB to change its thinking over the next two weeks.

The need for a degree of coordinated planning to control the rapid growth of the polytechnics and other colleges became apparent during the late 1970s, when the Department of Education and Science, which ultimately foots the bill for such institutions, decided to put an upper limit on the size of the repayment that it would make to local authorities, the titular sponsors. With the change in government in 1979, a scheme was promulgated that led eventually to the formation of NAB in February 1982. NAB's planning exercise is bound to be compared with that of the University Grants Committee in 1981, with the notable difference that NAB has declared openly the criteria it has employed. As well as the shifts of emphasis between subjects, NAB has tried to reverse the trend towards a concentration of higher education places in south-east England.

Mr Cecil Robinson, president of the National Association of Teachers in Further and Higher Education, has described NAB's proposals as "statistical mumbo-jumbo" that has no relation to wider educational issues. His association does not accept the need for an overall contraction, particularly when demand for higher education is increasing. Robinson says the NAB proposals take no account of the increasing demand for education

among those in middle age who have been made redundant. The association will be seeking declarations of support at this week's annual meeting of the Trades Union Congress.

The Committee of Directors of Polytechnics is more cautious in its response but expresses concern about the reduction in expenditure per student which will follow unless the Department of Education and Science has a sudden change of heart. But the polytechnics seemed to have fared better than smaller specialized colleges; two-thirds of them are now being consulted on student totals actually in excess of the number they originally asked for.

Tim Beardsley

French biotechnology

Making a splash

WHATEVER happens in the small print of the French research budget for 1984 (it will probably match inflation but is still being haggled over as part of the whole 1984 government budget), biotechnology seems unlikely to do badly. On Monday, the industry and research minister Laurent Fabius laid the first stone of a new building to be devoted to biotechnology at the Institut Pasteur in Paris and also launched an "international network for biotechnologies", previewed by the Versailles summit. The network will link France with Japan, Britain and Canada, and the whole European Community through Brussels: its secretariat will be in France.

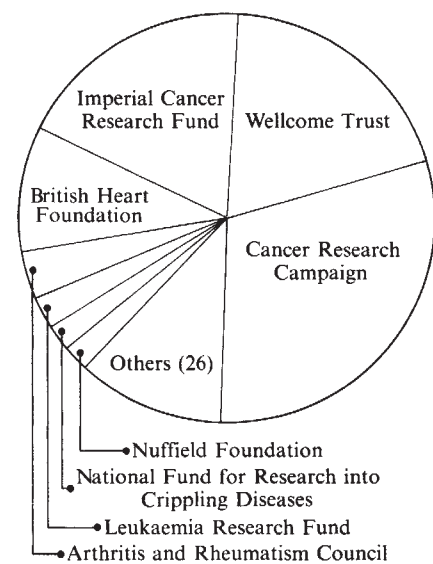
The new building will cost the state FF 60 million (£5 million). The Pasteur — which is private, but about 50 per cent state-supported — will spend a further FF 23 million (£2 million) to equip the six-floor building. The Pasteur's existing biotechnology group (G3, a syndicate which unites the Pasteur with other French research institutions) will occupy one-sixth of the nearly 3,000 square metres of useful laboratory space. Construction work should be completed during 1986, and although the building will be run by the Pasteur, the institute has promised to coordinate its research policies for users of the laboratories with the Centre National de la Recherche Scientifique, INSERM (the medical research council) and INRA (agriculture). The research will straddle the fundamental and the applied, oriented on the one hand to health, and on the other to industry.

As for the international network in biotechnology, that will establish communications among existing and future research centres, and "encourage" joint research programmes. An international committee will develop and control such programmes. President of the network will be Pierre Douzou, who runs the French biotechnology programme at the ministry of research and industry; the British government chemist, R. F. Coleman, will be vice-president.

Robert Walgate

Charities' research

THE total income of the 34 British medical charities that together make up the Association of Medical Research Charities was £115 million for 1983, of which just over £76 million was spent on research. This compares with the figure of £113.7 million allocated to the government-supported Medical Research Council (MRC), of which an estimated £100 million was to be spent on research. Source: Handbook of the Association of Medical Research Charities, 1983 and MRC Annual Report 1981-82. □



Nuclear power

Renewed urgency in Poland

LAST month Poland embarked on a plan to develop nuclear power stations — for the third time. An agreement signed on 27 July between the State Nuclear Physics Agency and the Association of Polish Electrical Engineers inaugurated a programme for the construction of nuclear power stations. Commenting on the agreement, Mieczysław Sowinski, chairman of the agency, remarked that the nuclear power industry in Poland was in a “highly unsatisfactory state”. This was something of an understatement — during the latter half of the 1970s the industry did not exist at all. The Atomic Energy Bureau was abolished in 1976 under Party Leader Edward Gierek, a former coal miner, and Poland's energy programme was based firmly on coal and lignite.

Even at the height of the Gierek era, however, a lobby in favour of nuclear power persisted. An agreement with the Soviet Union, for the construction of a nuclear power station at Zarnowiec, north of Gdansk, was signed in 1974. It was initially hoped that the first 440 MW VVER light-water reactor would be operational by 1984 but plans fell more and more behind schedule. By 1977, when the Soviet Minister of Electrical Power Development, Petr Neporozhnyi, visited Poland, there was talk of a nuclear generating capacity by the year 2000 that would equal Poland's total existing capacity. More immediately, plans were put forward for a second nuclear power station at Ciechocinek, in the Kujawy district, which would be sited on an artificial lake, to be created on the lower Vistula as part of a grandiose civil engineering scheme.

Meanwhile, theoretical work on nuclear energy went forward, and Poland began manufacturing nuclear power equipment for other Comecon countries. At the Zeran branch of the Nuclear Research Institute (transferred to the energy ministry when the Atomic Energy Bureau was dissolved) a team headed by Dr Zbigniew Jaworowski, found good reason for supporting nuclear power. They collected a considerable body of data showing that the radioactive fallout from conventional coal-fired power stations was greater than the estimated leakage from nuclear plants, and in September 1980, Dr Jaworowski was appointed head of a UNESCO commission on fallout problems.

The labour disturbances of 1980 caused some rethinking on coal. And nuclear power began to look more attractive when the search for oil beneath the Baltic Sea proved unproductive. In January 1981, it was announced that construction work at Zarnowiec would restart “by the end of the year”, but more than a year later, when a major fire broke out at the site (the damage was estimated at between 4 and 5 million zloty), all that had been consumed in the

blaze was the workers' barracks — nothing else had yet been constructed.

By this time, however, the need for nuclear power was officially considered “urgent”. A temporary government plenipotentiary for nuclear power, Professor Jerzy Minczewski, said in November 1981 that for the next four decades, nuclear power

would be the only way of bridging Poland's “increasingly evident” energy gap.

Present plans, according to Mr Sowinski, envisage a nuclear generating capacity of 8,000 to 10,000 MW by the year 2000. Construction of the Zarnowiec station has at last begun, and two 440 MW reactors are due to go into operation there in the late 1980s. At the same time, design work for the Kujawy station, which will have four 1,000 MW sets, is being given priority.

Vera Rich

Biotechnology index

Stocks sliding down

Washington

IN the face of the continuing slide of high technology stocks on Wall Street, Novo Industri was one of the few biotechnology stocks to buck the trend last month. A very favourable earnings report sent the stock up four points in a single day; more good news came with the approval on 30 August by the Food and Drug Administration of the human insulin originally developed by Novo Industri and produced in the United States in partnership with Squibb.

This product is made not by genetic engineering but by chemically altering pig insulin to produce a substance chemically identical to human insulin. The product will compete directly with Eli Lilly's genetically-engineered human insulin, which was introduced in the United States last month.

Lilly, however, has had a disappointing time with its human insulin; the *Wall Street Journal* last week said the product was off to “an excruciatingly slow start”. Lilly, which has long dominated the insulin

market with its animal insulin, says that it expects the human insulin to serve the 5 per cent or so of diabetics who are allergic to the animal product.

California Biotechnology, the genetic engineering company founded by E.F. Hutton, Inc., Professor Brian McCarthy (University of California, Irvine) and Professor John Baxter (University of California, San Francisco), announced last week that it will offer 1,600,000 shares of common stock in early October. The initial public offering price is expected to be \$12-14 per share.

The company currently manages a \$27.5 million research and development partnership for which it conducts research. It plans to develop products for human therapeutic use (specifically products for hypertension, immunosuppression and atherosclerosis) and for human diagnosis and animal therapeutic use. The sale of stock will result in a 30 per cent public ownership of the company, which was founded early in 1982.

Stephen Budiansky

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 26 August	Change
60 1/4	27 1/8	Pharmacia (Sweden)*	58 1/2	58	-1/2
23 1/4	14 1/2	Biogen (Switzerland)	16 1/4	14 1/2	-1 3/4
6 1/4	3	Bio-Logicals (Canada)	4 3/8	3 1/4	-1 1/8
16 1/8	7 1/4	Bio-Response (USA)	13 1/2	14	+ 1/2
19	11 5/8	Cetus (USA)	15 1/4	13 3/4	-1 1/2
15 1/2	8 7/8	Collaborative Research (USA)	11 1/2	10 1/2	-1
39 7/8	15	Damon (USA)	28 1/4	22 1/2	-5 3/4
34 1/4	16 3/8	Enzo-Biochem (USA)	28 1/2	25	-3 1/2
18 7/8	10 7/8	Flow General (USA)	12 1/4	11 1/8	-5/8
49 3/4	26 1/8	Genentech (USA)	45 1/4	43 1/2	-1 1/4
17 3/4	8 3/4	Genetic Systems (USA)	12 1/8	12 7/8	+ 3/8
23 1/4	12 7/8	Genex (USA)	18 1/2	19 1/2	+ 1
31	21 1/4	Hybritech (USA)	26 1/2	22 3/4	-3 3/4
23 1/4	14 3/4	Molecular Genetics (USA)	19	15 3/4	-3 1/4
22 1/4	13 3/4	Monoclonal Antibodies (USA)	15 1/2	15 1/2	0
65 5/8	42	Novo Industri A/S (Denmark)	57 1/8	61	+ 3 3/8

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. *Nature's* weighted index of biotechnology stocks stood at 232 on 26 August, compared with 238 a month earlier. Data from E.F. Hutton, Inc.

*Pharmacia (formerly A B Fortia) underwent a 5 for 3 stock split last month; prices have been adjusted to reflect this.

Out of sequence

SIR — I have followed the series of letters on nucleotide sequences with interest. Many of the difficulties encountered are easily explained by the following fundamental principles:

First law: DNA polymerase is much better than humans at proofreading. Corollary: The mutation rate is much higher in press than *in vitro* or *in vivo*.

Second law: Any sequence that is numbered is numbered incorrectly.

Third law: Any relationship between the length of sequence presented in a paper and the length actually determined is purely coincidental.

Fourth law: Identical sequences published by identical authors will not be identical. Corollary: If an explanation is given, the claimed differences and the actual differences will differ.

Fifth law: Journal articles, like mitochondria, use different genetic codes.

BOBBY BAUM

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Whaling quotas

SIR — S.J. Holt (*Nature* 14 July, p.110) states that evidence for the recovery of exploited species of whales comes mainly from modelling. Happily, there is much empirical evidence, from a large body of census information on the Californian grey whale *Eschrichtius gibbosus*, much more recent but well-documented information on humpback whales *Megaptera novaeangliae* especially in the north-west Atlantic, less-well-documented observations of recovery of blue whales *Balaenoptera musculus* in the Gulf of St Lawrence, and incipient recovery of several populations of right whales *Eubalaena* sp. in various sea areas, to which there was devoted a recent symposium under the aegis of the International Whaling Commission.

Two recent papers^{1,2} document the recovery⁴ of harp seals off eastern Canada from previous over-exploitation, since an effective quota was applied in 1972. One study is based on capture-recapture tagging, the second is a modelling exercise.

Opponents of a marine mammal fishery such as Holt argue that no exploitation is permissible unless cut-and-dried evidence is available on the competence of its management. It is probable however that no such evidence can ever be marshalled, since all ecological evidence is imprecise and therefore open to criticism.

The real test seems to be an adequate incentive for the government concerned to manage. But how can we measure incentive? I suggest an answer based on the involvement of the citizens of the country concerned. Thus both the Soviet Union, in the White Sea, and Canada, on its northern

Atlantic seaboard, have successfully managed (that is allowed to increase) their harp seal populations in recent years. Norway, in the western Barents Sea, has not. In winter, the Norwegian coast is ice-free all the way to East Finmark, while the White Sea and the Canadian north-east coast are ice-bound.

Both the Soviet Union and Canada therefore have large numbers of inshore fishermen, with small capital resources, who need the shore fishery of harp seals in winter *in perpetuo*. Since the harp seals leave the area in summer, summer fisheries are not affected by them. There is therefore a strong incentive for these governments to maintain the seal hunt.

Norway by contrast has a strong offshore fishery in the Barents Sea which views seals as competitors, while harp seals coming from the east get in the inshore fishermen's nets and are therefore a nuisance rather than a resource. Norway's seal hunt from Tromsø and Aalesund is a distant-water hunt which is more highly capitalized and perhaps therefore more easily changed to another enterprise; at any rate the fishing industry must outweigh the sealing industry in value of products.

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1. Bowen, W.D. & Sergeant, D.E. *Can. J. Fish. Aquat. Sci.* **40**, 728-742 (1983).
2. Roff, D.A. & Bowen, W.D. **40**, 919-932 (1983).

The patents jungle

SIR — The news item "French patents — Fanning fires of invention" (*Nature* 18 August, p.577) contains two distinct errors. First, in referring to France and Japan, the author states: "The patenting process in these two countries is roughly comparable. There is essentially no assessment or weeding out of applications in either country — anyone who applies (and pays) receives."

The Japanese system is in fact a far cry from the French. A substantive examination must be carried out in Japan, and if the applicant does not request it in the prescribed time, the application is deemed to have been abandoned.

The examination is far from being a mere formality. In fact, it often results in a rejection in the first instance. Japanese examiners are known to reject the application on the ground of obviousness after citing a textbook, or other well known reference, that does not even mention the problem to which the inventor has addressed himself. It is believed that Japan is particularly hard on foreign applicants and this may partly explain why only 30,000 patents were granted on foreign inventions in 1980, according to the author.

The second error is found in the statement: "However, the US patents system is

somewhat like the British — an application is examined and judged only if challenged." In fact British and US applications are never granted without examination. In the United Kingdom, both preliminary and substantive examinations must be carried out (at the request of the applicant), and it is essentially the result of the latter that determines whether a patent will be granted. The procedure is generally similar in the United States, except that once the application has been filed a single examination follows automatically within a certain time.

With regard to the imbalance between patents granted to national as compared with foreign applicants in West Germany, in accordance with the law of that country, an employer must make an early decision on any invention submitted to him by an employee in order to give the employee the opportunity to apply for patent protection himself if the submission is declined. This means that the employer is often compelled to accept the submission and file an application as a mere precaution. Not infrequently the subject-matter does not deserve to be called an invention. The West German patent system has indeed the reputation of being "rigorous", but it has nevertheless let through its fair share of trash. It follows that the number of patents granted to German nationals and the reputation of the German Patent Office are not necessarily good indicators that the said patents "probably represent a real national advantage".

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Kahn's legacy

SIR — I take exception to the laudatory tone of your "obituary" of Herman Kahn (*Nature* 14 July, p.102). He was, as you state, a man of exceptional gifts, but he used these gifts to provide a rationality for what I consider to be irrational ends. He never took cognizance of people, of ordinary persons, but of national states; he never discussed politics but wrote of governmental policies. He always wrote of categories and never mentioned the daily lives of people who are only on this Earth once. I consider Kahn essentially a coward, for he never wanted to acknowledge to himself or to others that his "unthinkable" was not nuclear war, but the murder of millions of innocent people. He, and others like him, were always play-acting, and their "realism" was in trying to make us believe their plays were the real thing. I, for one, and I consider myself a charitable man, do not regret his passing.

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Scientists' attitudes towards nuclear energy

S. Robert Lichter* & Stanley Rothman*

A large-scale survey of American scientists reveals strong support for continued nuclear energy development. Most regard thermal fission as one of the few resources capable of contributing significantly to our short-term energy needs and rate the risks as acceptable. They are critical of the performance of government and the public and they would prefer that public interest groups, the news media, and the general public exert less influence over nuclear energy policy.

THE importance of scientific opinion has increasingly been recognized and utilized by contenders for influence over nuclear policy-making. Yet no one really knows what the scientific community thinks about the risks and benefits of nuclear energy development. Despite the involvement of individual scientists in the debate over nuclear energy policy, no evidence has been available on the views of the entire scientific community.

Between March and October 1980, slightly over a year after the nuclear accident at Three Mile Island, we circulated a detailed questionnaire on energy issues to a stratified random sample of scientists listed in *American Men and Women of Science*. We sought to identify the views of both scientists in general and those working in energy-related disciplines.

We began by drawing a pure random sample of 1,000 names. With the assistance of several scientists, we then identified 71 disciplines that should yield some expertise in energy-related matters such as atmospheric chemistry, ecology, plasma physics and conservation (see panel). The random sample contained 163 scientists trained in these fields. We then randomly selected additional names within each energy-related field, until each discipline was represented by at least five names. This procedure, which yielded an additional 259 names, provides for more statistically reliable interdisciplinary comparisons, while substantially enlarging the pool of respondents with training in energy-related

matters. An overall response rate of 74 per cent was obtained.

We shall separately examine the views of the random sample from *American Men and Women of Science* ($n=741$) representing the entire scientific community and the stratified random sample drawn from energy-related disciplines ($n=358$).

Policy options

We then asked respondents to choose one of four different views on how the United States should proceed with nuclear energy development: (1) proceed rapidly with appropriate engineering safeguards; (2) proceed slowly and cautiously; (3) halt further development, while continuing to use existing plants; and (4) halt further

Table 1 Scientists' policy preferences on nuclear energy development (%)

	Random sample	Energy sample
Proceed rapidly	53	70
Proceed slowly	36	25
Halt development	6	4
Dismantle plants	3	1
No opinion	2	—
	100%	100%

development and dismantle existing plants.

The results (Table 1) show strong support for nuclear energy development, especially among scientists in energy-related fields. Among all scientists (the random sample), a majority of 53 per cent favours rapid development, and another 36 per cent would move forward slowly and

Disciplines included in energy sample

Air pollution, Alternative energy, Aquatic ecology, Architectural engineering, Atmospheric chemistry, Chemical engineering, Civil engineering, Climatology, Combustion, Conservation, Design engineering, Ecology, Energy analysis, Energy conversion, Energy engineering and technology, Energy policy, Energy sciences, Energy systems, Engineering, Engineering geology, Engineering management, Engineering mechanics, Environmental chemists, Environmental engineering, Environmental geology, Environmental health, Environmental management, Environmental medicine, Environmental toxicology, Exploration, Geology, Forest products, Fuel engineering, Fuel science

and technology, Futuristics, Geothermal energy, History of science, History of technology, Industrial engineering, Marine ecology, Marine engineering, Material science and technology, Metallurgical Engineering, Meteorology, Mining geology, Nuclear chemistry, Nuclear energy, Nuclear engineering, Nuclear medicine, Nuclear metallurgy, Occupational health, Ocean engineering, Petroleum microbiology, Physics, Plasma physics, Pollution biology, Pollution chemistry, Population ecology, Public health, Radiation ecology, Radiation genetics, Radiation physics, Radiological physics, Reactor physics, Science policy, Solar energy, Thermodynamics. □

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cautiously. A total of 9 per cent would halt further development, including 3 per cent who would dismantle existing plants. Among those whose disciplinary training should give them some expertise in scientific matters related to energy issues, 70 per cent endorse rapid nuclear development and 25 per cent slow and cautious development; only 5 per cent would preclude future nuclear options.

Scientists' broad support for nuclear energy development is underscored by their willingness to live with nuclear power plants. Fifty-nine per cent say they are willing to have nuclear power plants in their own communities, more than twice the 26 per cent who reject such sitings. The remainder are undecided. Once again, those with scientific training in energy-related matters emerge as stronger supporters of nuclear plants. Seventy-one per cent would be willing to locate a plant where they live, compared to only 19 per cent who reject the prospect.

We wished not only to determine scientists' policy preferences but also to examine possible reasons for them. Much of the questionnaire dealt with the role of nuclear power in the context of our energy needs, the problems associated with nuclear technologies, and the risks involved in exercising the nuclear option. Our findings suggest that scientists recognize some serious problems connected with nuclear energy, but that they believe these problems can be solved. In addition, they think the United States faces a severe energy crisis and they count nuclear power among those energy sources most capable of meeting the need, at least in the short run. Perhaps as a result, scientists believe that the very real risks associated with nuclear energy development are worth taking.

Energy outlook

As the oil shortage of the mid-seventies gave way to the glut of the early eighties, some commentators suggested that the energy crisis was over. The scientists we surveyed remain less sanguine. Seventy-two per cent regard the energy crisis as "very" or "extremely" serious, 22 per cent as "fairly" serious, and only 6 per cent as "somewhat" or "not at all" serious. Those in energy-related fields are slightly more pessimistic.

This assessment may be linked to the belief that energy usage will continue to increase during the coming decades, although at a considerably slower rate than during the fast growth period of the 1950s and 1960s. Despite widespread calls for conservation and, in some quarters, lowered living standards, only 11 per cent of the scientists predict that US energy usage will not increase during the next twenty years. The largest number, 44 per cent, foresee a 50 per cent increase in energy usage before the next century. Another 26 per cent forecast a doubling of energy usage, and one in ten believe our

energy use will at least triple during that time. On this matter, those scientists in energy-related fields differ only slightly from their colleagues.

If America is facing a genuine energy crisis, and our energy needs are likely to increase substantially during the next twenty years, what resources are capable of filling the gap? We asked scientists to assess the potential contribution of sixteen resources from biomass to windpower. The list included three separate forms of nuclear power: thermal fission reactions, fast breeder reactors, and fusion.

Table 2 lists the proportion of scientists who believe that each resource can make either a "moderate" or "large" contribution to meeting our energy needs. The ratings of the random sample and the energy-related sample are quite similar, with one exception. Coal and oil top both lists, with virtually all scientists arguing that these resources will continue to play a major part in our energy plans. Among the random sample, these are followed by natural gas and conservation. Nuclear power ranks fifth; two-thirds of the scientists grant thermal fission reactors a major potential role in our near term energy future.

Aside from these five, no other resource is seen by a majority of scientists as making more than a small contribution. So coal, oil, natural gas, conservation, and nuclear fission emerge as our primary potential means of combating the energy crisis, in the eyes of the scientific community.

Among scientists with expertise in areas related to energy, however, we find significantly greater optimism about the potential of thermal reactors. Almost nine out of ten rate this technology as a moderate or large contributor. This form of fission is ranked third by this group, just behind oil and ahead of natural gas and conservation.

This listing provides two clues regarding scientific support for nuclear energy. First, a large majority of scientists, and an even larger proportion of scientists in energy-related fields, see thermal fission as one of the few resources capable of contributing significantly to our energy needs before the year 2000. Second, scientists are relatively pessimistic about the short-term potential of "alternative" energy sources such as solar power and biomass, and of other highly touted technologies like synthetic fuels and shale oil extraction. Of all these options, only solar heat is accorded greater potential than an experimental and controversial form of nuclear energy, the fast breeder reactor.

It must be stressed that these results refer to the potential accorded each resource, without implying that a given potential should be realized. Thus, scientists might grant that nuclear energy can contribute to our energy usage without believing that it should do so. In fact, it appears unlikely that many would forego this option. Sixty-eight per cent of the random sample, and 85



Only 4% of energy specialists saw wind power as a moderate to large contributor to short-term energy needs. . .



. . . but 32% had high hopes for fast breeders

per cent of those in energy-related fields, rate the "present risks" of nuclear energy as "acceptable". These percentages correspond closely to the proportion of each group who accord thermal reactors a significant potential for helping to meet our energy needs. Only 8 per cent of the random sample and 5 per cent of the energy-related subsample rate the risks of nuclear power as "much greater than acceptable".

Prospects

The scientists we surveyed are by no means Pollyannas when it comes to assessing these risks. We presented them with a list of thirteen "problems" connected with nuclear energy and asked them to rate the seriousness of each. The list contains every major safety issue raised by critics of nuclear energy. The responses to this question are summarized in Table 3.

What do scientists' ratings of these problems tell us about their attitudes toward nuclear energy? First, large numbers do see some problems as quite serious. Their responses point to the training of plant personnel and the disposal of high level radioactive waste as matters of particular urgency.

Second, the problems that most concern them are rather different from those raised

Table 2 Per cent of scientists rating the potential short-term contribution of energy resources as moderate or large

	Random sample	Energy sample
Coal	96	99
Oil	88	90
Natural gas	81	87
Conservation	72	76
Thermal fission reactors	65	89
Hydropower	46	39
Solar heat	40	35
Fast breeder reactors	35	32
Synthetic fuels	32	32
Shale oil extraction	31	27
Passive solar	27	22
Fusion	24	12
Biomass	21	13
Solar electricity	16	7
Wind power	9	4
Tidal power	4	1

Table 3 Per cent of scientists rating problems connected with nuclear energy as "very serious"

	Random sample	Energy sample
Training personnel	61	47
High-level waste disposal	61	46
Proliferation	40	31
Consequences of accidents	40	26
Plant construction	33	24
Transporting waste	31	16
Plant design	30	21
Low-level waste disposal	29	18
Likelihood of accidents	28	17
Sabotage	27	19
Release of radioactivity	22	10
Decommissioning plants	21	12
Risks to workers	13	4

most often in the media and the public opinion polls. Few scientists express grave concern over matters related to the daily functioning of nuclear plants, the likelihood of accidents, or risks to plant workers and the surrounding community from radioactive contamination.

Third, scientists in energy-related fields are more sanguine than their peers in other disciplines. The random sample and the energy-related sample tend to agree in ranking the problems relative to one another, but the latter group rates every problem as less serious than does the former. For example, 10 of the 13 problems are rated as very serious by at least one quarter of the random sample. Among the energy-related group, only four problems elicit that much concern, and none is perceived as very serious by a majority.

These results raise an obvious question. Since large numbers of scientists agree that some of the problems connected with nuclear power are very serious, why are they so supportive of nuclear energy development? Our survey suggests most believe that we already possess the knowledge to solve the problems that exist. Three out of four scientists pronounce themselves at least "moderately confident" that we possess the knowledge to solve scientific and technical problems posed by nuclear

Table 4 Per cent of scientists rating factors as very important to nuclear development

	Random sample	Energy sample
Engineering/technical	86	85
Scientific/theoretical	56	45
Environmental	54	46
Economic	38	54
Moral	22	20
Political	18	29

Table 5 Scientists' ratings of groups who deal with nuclear energy: per cent rating each group's performance as good or excellent

	Random sample	Energy sample
Academic scientists and engineers	66	71
Industry scientists and engineers	64	76
Government scientists and engineers	51	58
Reactor technicians and personnel	40	48
Reactor owners and licencees	34	43
US government regulators	26	26
State and local authorities	15	14
The public	12	12
Congressional committees	8	10

energy, including 47 per cent who are "very confident" of our problem solving capacity. Only 26 per cent are only "somewhat" or "not at all" confident. Once again, scientists in energy-related disciplines are even more sanguine. Almost two out of three (65 per cent) are very confident that we already know how to solve these problems, and 86 per cent express at least moderate confidence.

Scientists' evaluations of particular problems are apparently related to their beliefs that we understand the phenomena involved. We asked respondents how well, in their opinion, scientists and engineers understand the problems of storing radioactive waste and the risks to workers in nuclear plants under normal conditions. Recall that scientists see the problem of high level waste disposal as the most severe problems of thirteen listed, while deeming the risk to plant workers as the least severe problem. It comes as no surprise, therefore, that they believe we understand the latter topic far better than the former. A minority (49 per cent) of scientists believe the problems of storing radioactive waste are understood at least moderately well, compared to over two-thirds (68 per cent) who ascribe that level of understanding to the risks incurred by nuclear workers. The same pattern is found among scientists in

energy-related disciplines, although they are more confident of their understanding of both problems.

Thus, despite their recognition of some serious problems, most scientists feel that today's nuclear power plants are relatively safe. We asked respondents to rate the current safety of nuclear plants on a scale ranging from one to seven, where a score of one was designated "very unsafe", four indicated "moderately safe", and seven, "very safe". The mean score for the random sample was 4.63, for the energy-related sample, 5.34. We also grouped respondents according to criteria that ranked a response of one or two as "very unsafe," three to five as "moderately safe", and six or seven as "very safe". On this basis, only 7 per cent of the random sample would classify nuclear plants as very unsafe, compared to 31 per cent who rate them as very safe. Among scientists in energy-related disciplines, 58 per cent rate nuclear plants as very safe at this time, and only 3 per cent as very unsafe.

A technical issue?

If the logic of many scientists' views on nuclear energy seems clear, the context of their position is no less apparent, as Table 4 indicates. Their views on the issue of nuclear development are guided primarily by technical and engineering considerations, and secondly by scientific, economic, and environmental concerns. They give short shift to either political or moral aspects of this issue.

Thus, scientists view the development of nuclear energy as primarily a technical matter. They grant the importance of theoretical and environmental considerations and, to a lesser degree, those of economics, but they feel that the politicians and moralists should give way to the engineers in planning our nuclear policy. Scientists in energy-related fields grant a somewhat more prominent place to the economic and political aspects of nuclear development, but they are united with their colleagues in placing technical and engineering considerations well above all others.

Policy-making

We asked respondents to rate the performance of nine groups who must deal with problems connected with nuclear energy. The proportion of scientists rating each group as "good" or "excellent" (as opposed to "poor" or "fair") is shown in Table 5. Results for the random and energy-related samples are quite similar. Both groups rate the performance of scientists and engineers highest, followed by that of industry-related groups, while government officials and the public receive the lowest ratings. Among the scientists and engineers, those in academia and industry are more highly rated than those in government.

It is hardly startling that these scientists in

should give high ratings to the performance of their peers. It is more noteworthy that they approve of industry researchers more strongly than those in government. In fact, the energy sample rates industry researchers above all other groups. This discrepancy cannot be accounted for by the character of the sample. It is made up primarily of academics (57 per cent of the random sample) with government and business-affiliated scientists represented about equally, at 20 and 23 per cent respectively. Their ratings of colleagues do reflect a general tendency to evaluate the performance of industry more highly than that of government, although no other group receives good or excellent ratings from a majority of either sample.

Moreover, scientists view with a jaundiced eye all major sources of government oversight of nuclear energy. Indeed they rank the performance of both the owners and operators of reactors above that of government regulators at all levels. Finally, their rejection of the general public's performance is especially notable. In fact, the public is the only group whose performance draws a "poor" rating from a majority of scientists.

Finally, we asked respondents who they believed should determine future American policy on nuclear energy. They were shown a list of ten current contenders for influence and asked how much influence each group should exercise over our nuclear energy development. Table 6 shows the percentage who would give a great deal of influence to each group.

Since these scientists view nuclear energy as primarily a technical issue, and given their ratings of their peers' past performance, it comes as no surprise that they assign the greatest influence to scientists and engineers in energy-related fields. Almost three-quarters (74 per cent) of the random sample and four-fifths of the energy sample see nuclear policy as a matter for the scientific specialists to decide.

If this result was not unexpected, the margin separating energy specialists from the next most preferred group is quite striking. Only 27 per cent of the random sample and 38 per cent of the energy sample would give a major role to government leaders. Since government leaders are necessarily the directors of American energy policy, this represents a notable lack of confidence in their stewardship. Indeed,

most scientists' unwillingness to entrust our nuclear future to government leaders is probably explicable only in light of their negative evaluation of past government performance. On the other hand, they would assign a greater role to government leaders than to any other group listed, aside from energy experts.

These scientists also see a role for popular participation in decision-making, although they distinguish between the "informed" public and the general public. (We included both designations at the urging of several scientists, who predicted that their peers would make precisely this distinction.)

These ratings suggest that the scientific community does not simply wish to

Table 6 Per cent of scientists who would give specified groups a great deal of influence over nuclear development

	Random sample	Energy sample
Scientists and engineers in energy-related fields	74	80
Government leaders	27	38
"Informed" public	24	27
Scientists and engineers in other fields	19	21
Public interest groups	15	5
Business leaders	12	23
"General" public	9	12
Labour leaders	3	9
News media	2	3
Religious leaders	2	1

arrogate to itself all decision-making on nuclear energy. Rather they tend to assign major roles to people they regard as knowledgeable. They rate scientific and technical experts above all others, to be sure, but they would also grant more influence to the informed layman than to scientists working outside the domain of energy.

Those surveyed reject the notion of a major role for the other contenders for influence — business and labour, the general public, "public interest" groups, religious leaders and the news media. The latter two groups in particular are disdained by the random sample and energy-related scientists alike. Scientists working in energy-related fields seem to be distinguished by their aversion to a major role for public interest groups, and for their willingness to accept a somewhat greater role for business leaders.

Conclusion

Our study does not purport to uncover the "truth" about the risks and benefits of nuclear energy, still less to suggest that policy should flow directly from the beliefs of the scientific community. We simply seek to document the views of scientists on this topic in a systematic fashion.

Within this limited sphere, our findings are rather clear and consistent. Insofar as the contestants in the nuclear debate wish to enlist the prestige of the scientific community in their cause, it appears that the pro-nuclear forces have the better of it. Large majorities of scientists, particularly those in energy-related fields, favour continuing nuclear development.

Given their assessment of our growing energy needs and the difficulty of meeting them, most scientists rate the problems involved in nuclear power as very serious, particularly those of training reactor personnel, high level waste disposal, proliferation, and our ability to deal with the consequences of accidents. At the same time, most are confident that they possess the knowledge to solve these problems. They believe that scientists and engineers have performed well in dealing with nuclear energy and should therefore continue to play a leading role. They are less than laudatory of the nuclear industry but they tend to reserve most of their criticism for the performance of government authorities and the public.

Just as their conclusions will not satisfy everyone, neither will the framework within which they make their assessments. Most scientists view nuclear development as primarily a technical matter, and very few hold either moral or political concerns to be central to this topic. Hence their unwillingness to allot much influence over policy-making to public interest groups, the news media, religious leaders, or the general public. Since numerous active and influential groups would take exception to their perspective, the scientific community is unlikely to have the last word on this increasingly volatile and emotional issue.

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Precursor phase of O₂ transition

Monolayer films of molecular oxygen adsorbed on graphite undergo two phase transitions — a lattice distortion and a magnetic ordering — and this presents calculators with a challenge.

WHEN is a phase transition to a disordered state a necessary prelude to a ferromagnetic phase transition involving long-range ordering of the spins in a lattice? The simple answer to this apparently paradoxical question is that the material must be molecular oxygen physically adsorbed in graphite as a two-dimensional layer and at a temperature of 11.5 K or thereabouts. The case has been sustained by a neat and sensitive measurement of adsorbed oxygen at low temperatures by R. Marx and B. Christoffer of the low-temperature laboratory of the University of Duisburg in West Germany (*Phys. Rev. Lett.* **51**, 790-794; 1983).

As a gas or liquid, molecular oxygen is familiarly known to be paramagnetic: the two outermost electrons have parallel spins and the net spin of the system is unity under all known circumstances. Single molecules of oxygen physically adsorbed on a graphite surface lie flat, with both atoms held to the surface, but as the occupancy of surface sites increases, oxygen molecules tend to stand on end. When there are 1.7 molecules of oxygen for each pair of possible adsorption sites on the surface, all of them are standing upright, perpendicular to the surface. The result is an array on a two-dimensional equilateral triangular lattice of molecules each carrying a magnetic moment parallel to the surface (and out of register with the underlying lattice). In this system a long-range ordering of magnetic spins should be possible, as calculations of the properties of two-dimensional Ising lattices have shown.

For several years it has been known that there is indeed a state of long-range magnetic order below about 11.5 K in which molecular oxygen behaves literally as a two-dimensional *antiferromagnet*, a consequence of the nature of the electron-exchange interaction between oxygen molecules which tends to align neighbouring spins in opposite directions. But as the lattice of the adsorption sites is triangular, it is not possible for the spins to be aligned so that all pairs of neighbouring spins are anti-parallel. Technically, the long-range magnetic ordering is "frustrated".

So how can a monomolecular film of molecular oxygen be a two-dimensional antiferromagnet? Only by some distortion of the two-dimensional lattice that removes the frustrating geometrical symmetry of the underlying equilateral triangular (or centred hexagonal) lattice. That there is,

indeed, such a distortion has been shown by neutron diffraction of molecular oxygen films (McTague, J.P. and Nielsen, M. *Phys. Rev.* **B19**, 3096; 1979). It consists of a distortion of the basic equilateral triangle (with sides of 3.27 Å) into an isosceles triangle in which one side is lengthened (to 3.43 Å) and the other two shortened (to 3.20 Å). In such a distorted two-dimensional array of oxygen molecules, the perfectly ordered antiferromagnetic state is that in which all spins point along the directions of the lengthened dimensions (in one sense or the opposite), and in which the rows of sites along the shortened dimensions of the lattice are occupied consecutively by alternating spins. Thus the exchange energy between neighbouring sites is a true minimum, and the net magnetic moment is zero, so producing an antiferromagnetic phase. The question that Marx and Christoffer tackle is whether the phase transitions consisting of lattice distortion and magnetic ordering occur together or separately.

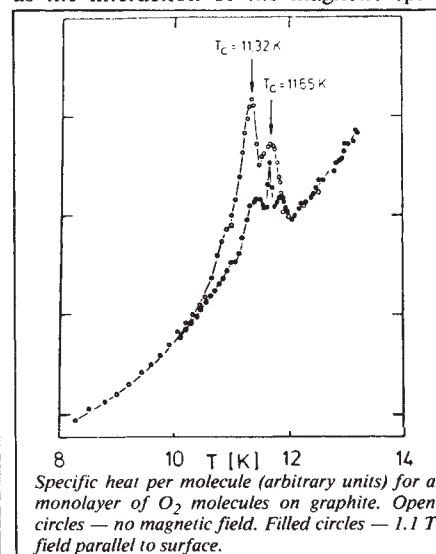
They have been able to measure the specific heat of oxygen adsorbed on graphite sensitively enough to show that there are indeed two phase transitions, at temperatures of 11.55 K and 11.32 K respectively. Part of the trick is to couple together two adsorption cells, one containing foil graphite "rolled like a carpet" and the other a stack of graphite sheets so as to obtain the specific heat of the adsorbed oxygen in fields which are either parallel or perpendicular to the graphite surface. The results (open circles in the diagram) show that when there is no field, there are indeed two distinct peaks in the plot of specific heat against temperature and, so, two successive phase transitions — not one.

Because the antiferromagnetic state is unattainable in the strictly symmetric triangular lattice, it is natural to infer that lattice distortion precedes the antiferromagnetic transition as the temperature falls. Marx and Christoffer have, however, proved the point by showing that the second transition is virtually abolished in a field of 1.1 T parallel to the adsorption surfaces (filled circles in the diagram) but not by a field perpendicular to its surface.

Such behaviour has previously been described only in three dimensions. Thus the material DyVO₄, antiferromagnetic at low temperatures, has two phase transitions at 3 K and 14 K which were interpreted as magnetic ordering and lattice deformation respectively (Sivardi re, J. and Blume, M.

Phys. Rev. **B5**, 1126-1134; 1972).

But how can a material which has never previously been near liquid helium "know" that its lattice must distort at some critical temperature to make possible antiferromagnetism at some lower temperature? Anthropomorphism is uncalled for, as the interaction of the magnetic spins



must be in part a determinant of the first preparative phase transition; their existence and configuration embody the "knowledge" of the system's low temperature fate.

Unfortunately, even the data Marx and Christoffer have produced on the specific heat of monolayer oxygen are not accurate enough to demonstrate that influence quantitatively. The authors do, however, say that the variation of specific heat with temperature in differently oriented magnetic fields shows that the lower transition is an Ising transition (order-disorder on a two-dimensional lattice occupied by two-valued scalar spins interacting only with nearest neighbours) and that the higher transition is a Potts transition (an elaboration of the Ising lattice.)

What this implies is that the properties of oxygen adsorbed on graphite should be almost exactly calculable, at least in the bottom left-hand (low temperature, low occupancy) corner of the phase diagram. Awschalom *et al.* have suggested (*Phys. Rev. Lett.* **51**, 586; 1983) that the rest of the phase diagram shows that when two regular layers have been filled, further adsorbed oxygen has the constitution of the bulk phases. This may make the challenge irresistible.

John Maddox

Genetic engineering

Drosophila takes off

from Andy Flavell

THREE papers published in the August edition of *Cell* together represent a landmark in genetic engineering¹⁻³. Each paper describes the introduction of a cloned gene into the germ line of the fruit fly *Drosophila melanogaster* by microinjection of recombinants based on the P-element vectors of Spradling and Rubin⁴. What makes the reports revolutionary is that in the main the inserted genes functioned normally.

In all three cases, the tissue specificity of the introduced genes — xanthine dehydrogenase¹, alcohol dehydrogenase² and dopa decarboxylase³ — was the same as in wild-type *Drosophila* and, in the case of dopa decarboxylase, the developmental profile of expression of the gene was very similar in five transformed lines to that of the control *Drosophila* stock. Most of the alcohol dehydrogenase transformants also behaved in the same way, although a few showed less fidelity. Furthermore, in all cases, one or at most a few copies of the recombinants became integrated and the level of expression of a particular inserted gene was always stably inherited. All this occurred despite the wide variety of new chromosomal locations into which the genes became integrated; in no case did the new gene occupy the normal locus.

P elements are transposons which are highly mobile in the *Drosophila* germ line when in the appropriate genetic background⁵. They were first cloned by Rubin, Kidwell and Bingham⁶ and first used as vectors for germline transformation by Spradling and Rubin⁴. All three series of experiments employ recombinants developed from a P element by these workers.

Thenew successes are particularly exciting because it will now be possible to study the DNA control signals which are required for regulation of gene expression in different tissues and at different developmental stages. *In vitro* mutagenesis (alteration of DNA in the test tube) can be used to modify the sequences around these (and other) cloned genes to see what DNA is required for their correct expression in *Drosophila*.

Germ-line transformation can also be used to study the effects of differing chromosomal environments on gene expression. For instance, one question addressed in the three papers is the mechanism of X-chromosome dosage compensation in *Drosophila*. *Drosophila* females are XX and males XY as in mammals. Both types of organism face the problem of controlling the expression of the genes on their X chromosomes, since females have twice as many X-specific genes as males. Unlike mammals, where one of the two X chromosomes is inactivated, *Drosophila* uses transcriptional

regulation to make each X-specific gene work twice as hard in the male as in the female fly. How this is effected at the molecular level is not yet known.

Two of the papers^{1,3} report that when an autosomal gene is put into an X chromosome its expression is at least partially dosage-compensated. This suggests strongly that the DNA surrounding the genes is responsible for the effect. One can easily envisage experiments using P-element-mediated transformation to find out whether X-chromosome DNA regulates the expression of adjacent genes. If specific X-chromosomal DNA sequences are responsible for dosage compensation then the sequences should cause a similar stimulatory effect on adjacent gene expression when introduced, via P elements, onto autosomes.

The correct expression of the genes introduced into the *Drosophila* germ line is in itself rather unexpected because related experiments on mice have usually failed to make cloned genes behave themselves when put back into their hosts. A lot of effort has been expended in introducing genes into mouse eggs (for review see ref. 7). The good news from these experiments is that DNA is quite readily incorporated into the chromosomal DNA of germ-line cells. The bad news is that, at least in the case of the β -globin gene, the inserted DNA is either not expressed at all or expressed inappropriately. Lacey and Constantini⁸ and others have introduced rabbit β -globin genes into mice but the introduced gene is not expressed in red blood cells; indeed, in one mouse line it is expressed in muscle (at very low levels) and in another, the testis, where the endogenous β -globin gene is silent (see ref. 7).

It is possible that the β -globin gene was an unfortunate choice as the results with recombinants based on a metallothionein promoter-herpes virus thymidine kinase fusion gene are more encouraging. When injected into mouse eggs these fusion genes are expressed in those tissues of the mice which produce metallothionein. Furthermore, the expression of metallothionein is responsive to the inducers zinc and cadmium (although not to glucocorticoids which enhance endogenous synthesis)^{9,10}. The difference between metallothionein and β -globin expression in mice may reflect a fundamental difference in gene regulation between highly tissue-specific genes (such as β -globin) and the less choosy genes (such as metallothionein) which are expressed in many tissues.

Further problems beset the experiments with mice. The number of genes inserted is highly variable, between a few copies and several thousand, and the same level of ex-

pression is sometimes not found in the offspring. In the long term these complications could prove useful for studying the factors which control gene expression but at present they make it very difficult to ascertain a control level of expression for the sort of *in vitro* mutagenesis work possible with *Drosophila*. And, of course, a last unfair advantage bestowed by nature on drosophilists is the generation time of the fly — about two weeks — as opposed to about nine weeks for the mouse.

The question of why introduced genes behave in *Drosophila* but not in mice is addressed in the three papers. The difference presumably either lies in the nature of the transduced DNA or of the DNA into which the gene is inserted, or reflects fundamental differences in the mechanisms of control of gene expression. If the first possibility is true it suggests that the P element can somehow insulate a gene from its environment or, alternatively, that it can 'activate' the gene so that it is affected by whatever factors determine differential gene expression. In this regard, it may be important that the vector carrying the genes is a mobile element, as one property which may be strongly selected during transposon evolution is the ability to function in a great number of chromosomal locations.

If the difference between *Drosophila* and mouse is actually something to do with the surrounding DNA then it may be that there is a greater proportion of the genome which is potentially expressible in *Drosophila* than in the mouse. In this case an introduced gene would more easily find a target site in which it could be transcribed. There is some evidence to support this hypothesis. First, Jaenisch's group¹¹ have observed that retrovirus genomes are often not expressed when inserted into the mouse germ line (5 out of 13 lines). They propose that the region into which the retrovirus provirus is inserted determines whether it will be expressed. Second, the *Drosophila* genome is twenty times smaller than the mammalian genome. The fly is obviously a simpler organism than a mouse but it seems unlikely that there are twenty-fold more genes in a mouse than in *Drosophila*. Is there any evidence for a lower, but finite number of inert sites in *Drosophila*? Because all three groups use the expression of injected genes as markers for successful transformation, the only flies which can answer this question are those in which more than one recombinant has been integrated. In all such cases look-

1. Spradling, A.C. & Rubin, G.M. *Cell*, **34**, 47 (1983).
2. Goldberg, D., Posakony, J. & Maniatis, T. *Cell*, **34**, 59 (1983).
3. Scholnick, S., Morgan, B. & Hirsh, J. *Cell*, **34**, 37 (1983).
4. Spradling, A.C. & Rubin, G.M. *Science*, **218**, 341 (1982).
5. Kidwell, G.M. & Kidwell, M.G. *Genetics*, **86**, 813 (1977).
6. Rubin, G.M., Kidwell, M.G. & Bingham, P.M. *Cell*, **29**, 987 (1982).
7. Williams, J. *Nature*, **300**, 575 (1983).
8. Constantini, F. & Lacey, E. *Nature*, **294**, 92 (1981).
9. Brinster, R.L. *et al.* *Cell*, **27**, 223 (1981).
10. Palmiter, R.D. *et al.* *Cell*, **29**, 701 (1982).
11. Jaenisch, R. *et al.* *Cell*, **24**, 519 (1981).
12. Cold Spring Harb. Symp. quant. Biol. **47**, 577 (1977).

ed at¹ each introduced gene functioned properly. Therefore, at least the majority of the *Drosophila* genome which is accessible to P-element insertion is capable of allowing gene expression.

The third explanation for the difference between mice and *Drosophila*, that of a qualitative difference in the mechanisms for regulation of gene expression, is supported by one line of evidence. Mammalian genomic DNA contains large amounts of methylated cytosine whose degree of methylation may be important in the control of gene expression¹². *Drosophila* contains little or no methylated DNA. Perhaps methylation is used by mammals, but not by *Drosophila*, as a method for selectively controlling gene expression.

It is obvious to the discerning reader that these arguments reveal the marked lack of understanding of the factors which control gene expression in multicellular eukaryotes. The torrent of work on *Drosophila* gene expression which can be expected to follow these papers will doubtless bring about major advances. For the present, at least, those studying gene expression in mice can only stare longingly over the fence at their more fortunate colleagues. □

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Astronomy

Galaxies in the early Universe

from N. Reid

THE completion of the La Palma observatory will provide British astronomers with an extensive array of instrumentation able to provide direct information on conditions in the early Universe. Partly with this in mind, this year's Herstmonceux conference on 'Observational Cosmology' was devoted to discussion of three topics: clustering of galaxies at different epochs; source counts and their interpretation; and the formation and evolution of galaxies themselves.

Since the pioneering work of Peebles, the spatial distribution of galaxies has been extensively used to test different scenarios of galaxy formation. M. Davis (University of California, Berkeley) described the constraints that can be set by the clumpiness of matter 'now', as revealed by the results of the Center for Astrophysics redshift survey. There are convincing indications from both this sample and others that the galaxy-galaxy correlation function is negative for separations of more than 20 Mpc, implying that galaxies are anti-correlated on such large scales. If true, this restricts the exponent of the initial power spectrum of density perturbations in the early Universe to positive values — reassuringly consistent with the value of 1 predicted by GUTS.

There are two kinds of explanation for the clustering of galaxies. On the one hand is the hierarchical picture, largely developed by Peebles, where galaxies, descendants of isothermal fluctuations in the early Universe, are the building blocks for clusters. On the other hand, the 'pancake' theory, primarily due to Zeldovitch, envisages adiabatic, rather than isothermal, fluctuations, smoothing out density variations on all save the largest scales ($10^{12} M_{\odot}$) until matter and radiation decouple. Thus clusters form from the top down in the latter theory. Both scenarios are open to numerical modelling, and

Davis described a number of pancake-type simulations currently being studied. While these adequately reproduce the observed spatial distribution of particles, models based on the pure Zeldovitch approach have velocity fields that are too vigorous, and imply a neutrino mass of ~ 60 eV and galaxy formation at a redshift of $z \sim 1$. We might expect to have positive observational evidence, by now, of both these predictions. However, combining these two approaches, Dekel (*Astrophys. J. Lett.* **261**, L31; 1982) has proposed a modified picture where galaxies form first from isothermal perturbations in pancake structures, with subsequent non-dissipational clustering on smaller scales. Results from these models are more encouraging.

Dekel's scenario — which predicts an increase of clustering at relatively recent epochs — was also favoured by Sargent (Caltech) from correlation analysis of quasar absorption-line systems. These observations probe galaxy clustering at early times: metal lines are interpreted as intervening galaxies; Lyman α systems as intergalactic hydrogen clouds (LACs) of dimensions $R < 200$ kpc. Neither line systems give evidence for significant clustering on extragalactic scales over $1.7 < z < 3.3$; contradicting the predictions of the isothermal hierarchical collapse theory, unless it is assumed that LACs are disrupted by, and therefore not associated with, galaxies. P. Kronberg (University of Toronto) described Faraday rotation observations towards a number of quasars, which indicate that typical magnetic field strengths in LACs are less than a few tens of microGauss — although two systems with $z_{\text{abs}} \sim z_{\text{QSO}}$ have milliGauss fields. Further observational studies of clustering were presented by S. Beard (University of Edinburgh), using UKST objective prism plates, and by S. Eales (Mullard), from charge-coupled device observations of the

environment of 3C sources.

Source counts, at the simplest level the variation in number density with apparent flux, are a powerful test of luminosity and/or density evolution, and M. Schmidt (Caltech) reviewed the results from analyses of optically selected samples of quasars. In particular, the redshift distribution of quasars in the Palomar-Green survey shows that the co-moving density increases by a factor of 200 by $z \sim 2$, with evolution strongest for the high (intrinsic) luminosity sources. There is however growing evidence from deep ($B > 21$ mag) surveys for an absence of quasars — at least, of quasars with strong emission lines — at redshifts $z > 3.5$. This is the redshift cutoff originally proposed by Osmer. H. Marshall (Center for Astrophysics, Harvard) described how analysis of a complete sample of fainter quasars, again optically selected by UV excess and of known redshift, supports the requirement for both density and luminosity evolution.

Radio-source counts have long played a major part in observational cosmology. J. Wall (RGO) outlined results from two contrasting surveys; of the 231 brightest sources at 2.7 GHz, and of a deep small-field VLA survey. Dividing the first sample into steep and flat spectrum sources, both of these groups show similar evolution at low flux densities, but the bright steep spectrum objects have a euclidian distribution, suggesting significant contribution from nearby low-luminosity sources. The deep survey, at 5 GHz, includes only 15 objects but these represent a substantial excess over the number predicted by extrapolating models which fit at brighter fluxes. J. Peacock (Royal Observatory Edinburgh) gave a more detailed description of modelling the radio-source distribution in the two-dimensional planes defined by redshift, radio power and spectral slope. Preliminary results show interesting indications of a redshift cutoff.

Galaxy evolution can be constrained by combining source counts at different wavelengths. J. Allington-Smith (University College London) and P. Katfert (University of Leiden) described infrared and optical observations of complete samples of radio galaxies, while R. Shafer (Institute of Astronomy, Cambridge) discussed the constraints set by X-ray observations on the evolution of active galaxies. T. Shanks (University of Durham) presented field galaxy counts derived from COSMOS measurements of blue and red UKST and AAT plates. While there is little evidence of luminosity evolution in the red, the slope of the blue counts suggests the galaxies are brighter by 2–3 mag in the (rest frame) UV.

Obviously the presence of extra blue flux suggests extensive star formation, and J. Silk (Institut d'Astrophysique, Paris) emphasized the importance of dissipative processes in galaxy formation in explaining many of the observed properties of galactic systems. Adopting the hypothesis that

galaxies form through coalescence of smaller clouds, the pancakes of the adiabatic theory become natural reservoirs of internally supported low-mass gas clouds which form clusters of galaxies. Galaxies and clusters form at the same (relatively recent) epoch in this picture, with gaseous infall continuing into the present. This framework also allows gas-rich systems to survive until the present epoch without significant star formation. R. Terlevich (Institute of Astronomy, Cambridge) described observations of violent star formation in these HII galaxies, showing that the metallicity is correlated with the mass/light ratio, the effective temperature of the brightest stars and the total UV flux. These correlations are most plausibly explained as changes in the slope of the high-mass star initial-mass function, in the sense that it is flatter at lower metallicities.

Like the HII galaxies, ellipticals appear simple single-population systems at first sight. Recent dynamical studies have, however, shown this to be far from true. C. Jenkins (RGO) showed that powerful radio galaxies appear to have significant rotation, along the minor axis, suggesting that these may be tri-axial systems. S. Phillips (University College, Cardiff), considering the radio properties of all Hubble types, showed a loose correlation between radio luminosity and surface brightness.

S. Lilly (University of Edinburgh) discussed infrared (IR) and optical observations of the more distant 3C radio galaxies. While the IR colours are well fitted by passive evolution of a coeval population, the optical to IR colours required active star formation. However, the scatter in the latter indicates idiosyncratic variations from one galaxy to another, rather than the presence of a 'new' population. This is obviously important for population synthesis models, such as those described by G. Bruzual (University of Durham). By and large, ellipticals are well represented by models with a single burst of star formation, although an extant problem is the substantial UV rising branch observed in most such galaxies. M. Capaccioli (University of Padova) presented a review of IUE observations which show there to be little correlation between the UV flux and total luminosity. This flux, indicative of temperatures of $\sim 30,000\text{K}$, is generally credited to horizontal branch stars or continuing star formation but, discussing the impact of binaries on population synthesis models, A. Collier (RGO) pointed out cataclysmic variables as another possible source. Finally, R. Ellis (University of Durham) suggested three areas that studies of galaxy evolution should focus on: an improved determination of the "standard" energy distribution of the different Hubble types; optical and IR studies of cluster galaxies, extending down the luminosity function and to higher z ; and more extensive field redshift surveys, to $R \sim 22$.

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Mathematics

Novel techniques in bifurcation theory

from Ian Stewart

BIFURCATION theory is the study of how solutions to differential equations can change as the values of numerical parameters vary. Traditionally a parameter value is a bifurcation point if the number of solutions (with given boundary conditions) changes there. For example, the equations describing an elastic rod under compression have a single solution (the unbuckled state) below a certain threshold of compression, and three solutions (buckle up, buckle down and an unstable unbuckled state) above threshold. Another important example is the Hopf bifurcation, in which a stable steady state becomes unstable and throws off a branch of oscillatory solutions. Many important natural phenomena have been modelled successfully by bifurcations of differential equations.

A major difficulty is to find conditions sufficient to guarantee bifurcation. The traditional approach has been heavily analytical, with the emphasis placed on delicate functional analysis. A forerunner of recent developments was the introduction of topological methods, notably degree theory, by Krasnoselskii¹ in 1964. In the last five years several novel and powerful techniques have been imported from pure mathematics, and it is likely that these will produce fundamental changes in the way bifurcation problems are tackled in the future.

Singularity theory, an offshoot of René Thom's catastrophe theory, appears in the generic bifurcation theory of Chow, Hale and Mallet-Paret². A related and highly significant development is the imperfect bifurcation theory of Golubitsky and Schaeffer³, which adapts ideas from catastrophe theory to the analysis of bifurcations. The result is a rigorous method for detecting and classifying bifurcations of steady states, together with a sophisticated technique, unfolding theory, for analysing perturbations. The results generalize to the important case where symmetries are acting⁴, and to oscillatory systems exhibiting a degenerate version of the Hopf bifurcation⁵. Applications include an explanation of mode-jumping in the buckling of a rectangular plate⁶, the explosion peninsula in chemical reactions⁷, the behaviour of Bénard convection cells in the spherical annulus⁸ and the plane⁹, and periodic wavetrains in the Hodgkin-Huxley nerve impulse equations¹⁰.

Topological dynamics, the creation of Stephen Smale and his co-workers, is increasingly being applied to bifurcations. Smale¹¹ generalized the definition to allow changes in the topological type (and not

just the number) of solutions, a far-reaching idea now bearing fruit in the theory of chaotic systems. These are deterministic systems that exhibit what appears to be random behaviour. Important contributions have also been made by the Russian school of Vladimir Arnol'd.

The latest import from modern pure mathematics is algebraic geometry. A recent paper of Buchner, Marsden and Schechter¹² proves a very general (and computable) sufficient condition for bifurcation, which extends several of the standard theorems. Their argument makes essential use of the 'blowing-up' construction developed by algebraic geometers to resolve (get rid of) singularities (bad points) in algebraic varieties (solution sets of polynomial equations). A simple version of blowing-up is to replace a cone by a cylinder, by expanding the vertex point into a circle. The modern version is highly abstract and a good deal more sophisticated, and was developed as a technical tool within pure mathematics. The results of Buchner *et al.* apply to a wide range of bifurcation problems, including multi-parameter problems and multiple eigenvalue problems, and reveal the importance of a certain regularity condition on the leading terms of the equation. Several extensions and applications are promised.

Pure mathematics is sometimes criticized for a lack of attention to application. While it is no doubt true that individual mathematicians do not always bear application in mind, it appears that the results obtained by them may nonetheless prove useful in applied science. Powerful mathematical techniques remain powerful, no matter what their origins may be; and the origins in no way determine the areas to which they may be applied. □

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Palaeontology

No consensus on *Archaeopteryx*

from Michael J. Benton

IN the last few years there has been a resurgence of interest in the oldest known fossil bird, *Archaeopteryx*. Contention has focused on its functional anatomy (could it fly?) and its relationships (is it the ancestral bird?; indeed, is it a bird?; did birds originate from dinosaurs or from some earlier stock?). The first specimen of *Archaeopteryx* was collected in 1861, and since then the total has risen to five specimens and a feather. All come from the Upper Jurassic (~150 million years ago) Solnhofen Plattenkalk of Franconia, West Germany. The most recent work on the 'London' specimen¹ reflects the inconclusive nature of the arguments surrounding this contentious creature.

Ostrom^{2,3} has expressed the view that *Archaeopteryx* was a cursorial terrestrial predator that could not fly, primarily because it lacked the deep breast-bone that provides an anchor for the powerful flight muscles in living birds. However, Olson and Feduccia⁴ have argued that this is not the case, and that the important downstroke muscles in *Archaeopteryx* attached to its well developed furcula ('wish-bone').

The feathers offer further evidence. They are excellently preserved as impressions in fine limestone and this has permitted a detailed description of those of the 'Berlin' specimen (ref. 5 and see the figure). The feathers are asymmetrical⁶, with longer hairs on one side of the quill than on the other. This asymmetry has an aerodynamic function and is characteristic of flying birds: flightless birds have symmetrical feathers.

In the new work that has recently been done on the braincase of *Archaeopteryx* by Whetstone¹, the cranium of the 'London' specimen has been removed from its limestone slab and painstakingly prepared by mechanical means. This has shown that the skull is much broader and more bird-like than had been thought⁷. Details of the braincase and associated bones at the back of the skull seem to suggest that *Archaeopteryx* is not the ancestral bird, but an offshoot from the early avian stem. The exact relationships of the quadrate and squamosal bones, which link the braincase with the jaw articulation, are uncertain, but they appear to be more primitive than modern birds in some respects. In other respects, *Archaeopteryx* is advanced: the top of the quadrate has a single articulation (rather than a double one, as in other archosaurs and in modern birds), the blade of the scapula (shoulder blade) attaches to one of the ribs and the metatarsals (foot bones) are fused in a transverse plane.

The relationships of *Archaeopteryx* and the origin of the birds are controversial. In a recent review, Thulborn and Hamley⁸

identified seven hypotheses concerning the affinities of *Archaeopteryx*, three of which appear to have supporters at present. The work of Whetstone¹ just described does not so far provide strong evidence for or against any of these ideas, and further analysis of the material is necessary.

Walker^{9,10} has suggested that crocodiles and birds (and thus *Archaeopteryx*) derived from a common ancestor among the thecodontians (the basal reptile stock that gave rise to crocodiles and dinosaurs). His evidence depends on resemblances between the braincase and skull of recent crocodiles and birds, as well as bird-like features of a possible early crocodile called *Sphenosuchus*. Whetstone and Martin¹¹ supported this view with further evidence from the braincase of birds and crocodiles. Both groups were said to share an homologous specialized opening in the wall of the inner ear region (the fenestra pseudorotunda) and homologous pneumatic spaces in the bones around the middle ear. Unfortunately, few of these features could be seen in *Archaeopteryx* and there is no

compelling evidence of an advanced character(s), shared between *Archaeopteryx* and some, or all, crocodiles, but absent in the thecodontians and dinosaurs^{8,12}.

Ostrom¹², on the other hand, has proposed the derivation of birds from the dinosaurs on the basis of many characters, of the limbs in particular, shared between *Archaeopteryx* and certain theropod dinosaurs (bipedal meat-eaters). He proposed an evolutionary sequence from thecodontian to theropod to *Archaeopteryx* to modern bird. Walker¹³ has argued that Ostrom's interpretation of the pelvis of *Archaeopteryx* is wrong and makes it seem more dinosaur-like than it really was. In modern birds, the pubis runs backwards below the other two pelvic bones, while in theropod dinosaurs the pubis points forwards. Ostrom¹² reconstructed the pubis of *Archaeopteryx* as pointing straight down — an intermediate position; while Walker¹³ reconstructed it in a more bird-like backward orientation. Further, Martin *et al.*¹⁴ have argued that the ankle and teeth of *Archaeopteryx* could not be derived from those of theropod dinosaurs. Theropod dinosaurs and birds have an ascending process from the ankle in front of the tibia (shin bone), but it is apparently not homologous in the two groups, and the teeth of primitive birds are more crocodile-like than dinosaur-like. Finally, Tarsitano



The Berlin specimen of *Archaeopteryx lithographica* found in 1877 near Eichstätt, Germany. Preservation of feather impressions established these as the remains of a true bird, despite the fact that the skeletal anatomy is more like that of theropod dinosaurs than that of modern birds. Scale bar, 100 mm.

and Hecht¹⁵ criticized various aspects of Ostrom's hypothesis, and they considered that he had misinterpreted the homologies of the limbs of *Archaeopteryx* and theropods. Thulborn and Hamley⁸, reviewing all the criticisms, however, have concluded that they are without foundation (incorrect interpretations, inconclusive evidence, persistence of primitive characters), and that they do not "seriously weaken the hypothesis that *Archaeopteryx* is closely related to theropod dinosaurs".

The third current view of the relationships of *Archaeopteryx* has been given by Tarsitano and Hecht¹². They revived an old idea that *Archaeopteryx* is a member of a distinct lineage that arose from the thecodontians and has no direct relationships with crocodiles or dinosaurs. Their evidence consists of a resemblance between the coracoid (a bone of the shoulder girdle) of *Archaeopteryx* and some thecodontians, as well as arguments against the 'crocodile' and 'theropod' hypotheses. Thulborn and Hamley⁸ take the view that this hypothesis was selected merely by a process of elimination, but Hecht and Tarsitano have more recently reaffirmed their viewpoint¹⁶.

There are thus three strongly held views on the relationships of *Archaeopteryx* — that it is related to crocodiles^{9-11,13,14}, that it is related to theropod dinosaurs^{8,12} and that it is related to thecodontians^{15,16}. It might seem to be an easy question to solve in view of the relatively well preserved specimens of *Archaeopteryx* and of thecodontians and early dinosaurs and crocodiles. But the arguments rest on interpretations of the anatomy of *Archaeopteryx* and related forms, and on modes of interpreting the data — whether by seeking general resemblances and ancestors, or in attempting a strict cladistic analysis of sister-group relationships. A rumoured sixth skeleton of *Archaeopteryx*¹⁷ may offer new light on its anatomy. Interest in the 'early bird' or 'Urvogel' is so strong that a conference devoted to it is to be held in Eichstätt, West Germany, in September 1984. □

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Geophysics

Magnetic reversals from a submersible

from J.R. Cann

THE Vine-Matthews hypothesis¹ is 20 years old this year, and coincidentally a paper has just been published by Macdonald and co-workers² which represents a decisive step in the affirmation and development of the hypothesis. In case you need reminding, the hypothesis is that the lineations observed on magnetic anomaly maps of the ocean basins³ can be explained as corresponding to strips of alternately normally and reversely magnetized sea floor. These strips were formed, it is argued, in a narrow zone along the crests of the mid-ocean ridges as the ocean floor spread apart continuously while the Earth's magnetic field periodically reversed. The ocean crust created, and especially the upper part of it, made up of submarine basalt lava flows, thus acted as a record of the field reversals.

A large amount of evidence has now accumulated that this hypothesis is a good model for the ocean floor, but the evidence has always been essentially indirect, and some scientists have contrived still to maintain their disbelief. The new paper does two things. It provides concrete support for the Vine-Matthews hypothesis through direct observation of a magnetic reversal on the ocean floor, and it also contributes important evidence about the creation of crust at mid-ocean ridges. The latter topic is especially important at present given the great interest in the black smoker springs and ore deposits of very young ocean crust⁴.

Working from the US submersible *Alvin*, Macdonald and co-workers used a vertical magnetic gradiometer made from two vertical-component fluxgate magnetometers spaced 30 cm apart to find the polarity of magnetization of individual outcrops of basalt (usually single pillows) at nearly 300 places on several transects across a predicted reversal boundary on the sea floor. This boundary corresponds to the most recent major reversal of the Earth's field at 0.7 Myr (the Brunhes-Matuyama boundary). A previous survey with a near-bottom towed vehicle had enabled them to calculate where the reversal boundary was likely to occur. The submersible measurements found a sharp boundary as expected, on one side of which (the side nearer the mid-ocean ridge crest) the outcrops of basalt were all normally magnetized, and on the other all reversely magnetized. This is clearly the outcropping of a Vine-Matthews stripe edge. In places it was covered by a sediment pond, but in others it appeared as a flow front of normally magnetized lava overlying reversely magnetized lava (thus showing the normal-

ly magnetized lava to be younger, as indeed it ought to be). Near the boundary some of the outcrops are very weakly magnetized, and these may be of lava erupted while the magnetic field was in the course of reversing.

The experiment is a particularly elegant demonstration of the Vine-Matthews hypothesis because it is very direct and can be repeated in principle anywhere in the oceans where a reversal boundary is not covered by sediment.

But what about crustal formation? The important evidence here is that the reversal observed in the outcrops is systematically displaced 250–500 m away from that calculated from the near-bottom survey, in a direction opposite to that in which the mid-ocean ridge crest lies. This had been anticipated by some modellers⁵, who considered from the evidence of ophiolite complexes (slices of ocean crust thrust above sea level during mountain building) that the zone of fissures from which lava is erupted must be about 50 m wide, much less than the distance that lavas flow away from the fissures (0.5–1 km)⁴. Macdonald and co-workers point out that the reversal from the near-bottom survey should correspond approximately to the position where the reversal is about half-way buried in the lava pile. This line in turn, if the fissure zone is indeed very narrow, should be displaced from the outcrop of the reversal by half of the lava flow length in the direction towards the mid-ocean ridge crest, which corresponds well with the observations.

Other modellers^{6,7}, whose experience had been conditioned by magnetic observations in deep-sea drilled holes on the Mid-Atlantic Ridge, had expected a much more irregular relationship. Such an irregular structure may be characteristic of the highly rifted (and generally slower spreading) ridges such as the Mid-Atlantic Ridge, while the narrow zone of fissuring may only be found on the less rifted ridges such as the East Pacific Rise where the experiment was performed. It is clearly necessary to resolve this question by repeating the experiment in the Atlantic. □

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Molecular biology

CACA sequences — the ends and the means?

from John Rogers

AMONG the various classes of dispersed repeated sequences in eukaryotic genomes, the simple sequence (GT)_n or (CA)_n has long looked like a poor relation. It has excited interest only recently, because of its propensity to adopt the left-handed Z-DNA form¹⁻³, which some researchers suspect is involved in gene regulation. Pending a decision on the functional relevance of Z-DNA (and of authors' titles which invoke Z-DNA to describe these dispersed repeats), I suggest here that (CA)_n sequences deserve attention on other grounds. First, they seem to represent insertions, which I suggest may have their origin in the dynamic activity of chromosome ends. And second, this activity seems to be only one of a variety of cutting and joining processes that centre around C-A dinucleotides.

It is now clear that (CA)_n sequences are widespread in a broad range of eukaryotic genomes. The first to attract attention was one of variable size in the large intron of the human γ -globin genes, for which Slightom *et al.*⁴ proposed an origin as the endpoint of a gene conversion event. (Like several other examples, this block also includes a minor repeating segment of a different dinucleotide, retaining the pyrimidine-purine alternation.) Other (CA)_n blocks have now been recognized in the globin gene clusters⁵⁻⁸, in a c-myc gene⁹, in some sequences of the dispersed *Alu* family^{10,11}, and in or around genes of the actin^{11,12}, immunoglobulin-V^{3,13} and histocompatibility¹⁴ families. By hybridization with (CA)_n · (GT)_n probes, it has been shown that these sequences are widespread in the genomes not only of mammals but also of frogs, slime mould and yeast^{6,11,12}.

That many of these blocks are insertions is suggested by the fact that many, though not all, of them are flanked by terminal repeats of 6–8 base pairs (bp)^{4-7, 12-14}. One even has an imperfect 24-bp terminal repeat⁶. Another sequence, from the mouse immunoglobulin C_H3 intron, can be added to this list:

CCTAACTA TT(CT)₂₉(CA)₃₀(GA)₄G-CCTAACTA

Terminal repeats are generally taken as *prima facie* evidence of insertion at a staggered break, in the manner of transposons, foldback elements and 'retrotransposons' (presumptive cDNA insertions such as *Alu*). Proof that some (CA)_n blocks are insertions comes from Gebhard's and Zachau's¹⁵ discovery of a block directly inserted into a member of the B2 retroposon family, with terminal duplication of only 2–3 bp.

How might these blocks be inserted? I

propose elsewhere a model whereby the insertion of a retroposon is initiated at a staggered break in the target DNA, by 3'-polymerization of (GT)_n or T_n onto one side of the break before cDNA synthesis. Possibly some events of this type can polymerize (GT)_n before filling in and re-sealing the nicks. Alternatively, since eukaryotic cells possess promiscuous ligation activities, some insertions of DNA at staggered breaks may result from integration of (GT)_n or (CA)_n fragments that happened to be present in the cell.

What might be the source of (GT)_n fragments or of a (GT)_n polymerization mechanism? The most promising source seems to be the ends of the chromosomes. Walmesley *et al.*¹⁶ showed that (GT)_n · (CA)_n tracts occur near the ends (telomeres) of chromosomes in yeast. Moreover, when minichromosome ends from the ciliate protozoan *Tetrahymena*

were used as termini for a linear plasmid in yeast, these termini also acquired (GT)_n · (CA)_n tracts during replication in yeast¹⁶.

We do not know the role of (CA)_n sequences at telomeres. The major telomere function must be to preserve the terminal sequences during DNA replication, as reviewed in these columns¹⁷, and this may involve nicking and resynthesis of DNA at specific sites. (CA)_n or related sequences may provide such sites, both in ciliates and in yeast and (since these organisms come from different kingdoms) perhaps in a wide range of eukaryotes. As evidence for this, *Tetrahymena* telomeres themselves carry related repetitious sequences (C₄A₂)_n, with nicks at some of the A-C positions *in vivo*¹⁸, and the same pattern of nicks is seen when these telomeres are grown in yeast¹⁹. In *Tetrahymena*, these 'telomeres' are only generated in the macronuclear rDNA, when a single internal C₄A₂ sequence is cut and then amplified at the free 3' end²⁰. Similarly, other ciliates such as *Oxytricha* acquire (C₄A₄)_n sequences on macronuclear DNA termini, in this case at the 5' ends²¹. The remarkable observation²² that *Trypanosoma* telomeres grow steadily longer during vegetative growth of the protozoan suggests that these telomeres,

THE INTERNATIONAL BUREAU OF WEIGHTS AND MEASURES

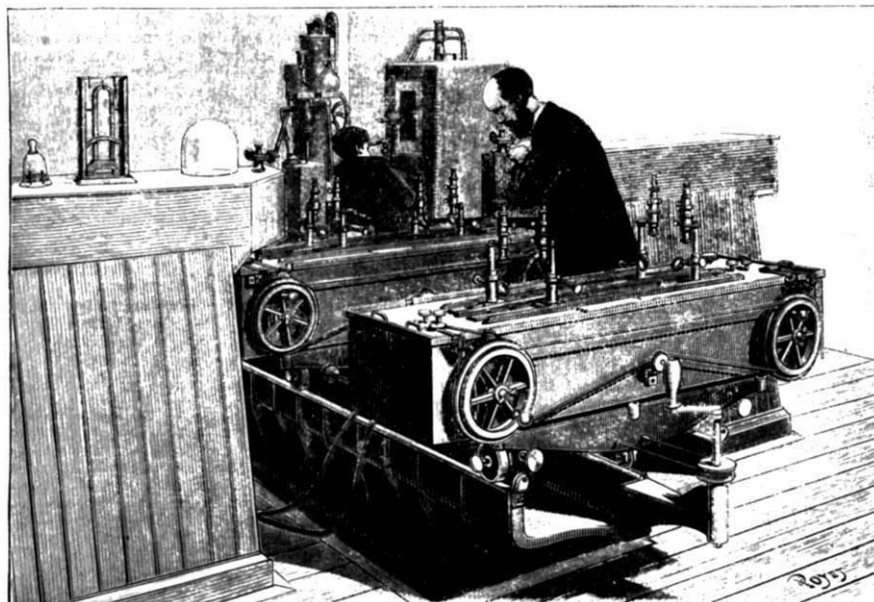
As the result of an International Convention held on the 20th May, 1875, an International Bureau of Weights and Measures has been created in Paris with the object of securing an international metric system.

The instruments belonging to the section of linear standards or metres, are called "comparateurs". A comparateur for metres *à traits* is essentially composed of two microscopes solidly fixed and immovable, provided with micrometers under which, by a peculiar mechanism, the two rules it is desired to compare with each other can be successively introduced.

One of the comparateurs represented in the accompanying diagram (Fig. 1) is designed for the measurement of expansions. We find two microscopes with fixed micrometers and a carriage moving on rails. The carriage contains two

distinct boxes or troughs; each at a distance of about one metre from the other. The two rules to be compared are placed one in each of these troughs. They are thus to some extent independent of each other, and may therefore be introduced at different temperatures. To measure the expansion of one rule, you place it in one of the troughs, and in the other trough the other rule called "*de comparaison*". This latter, so long as the process of determination lasts, is maintained at an invariable temperature, while the other is alternately cooled and heated throughout the series of consecutive experiments between sufficiently extended limits. The latter rule then alternately contracts and expands, and in the case of each particular experiment you compare the length the rule has reached at the temperature to which it is then subjected, with the constant length of the "*de comparaison*" rule.

From *Nature* 28, 464, 13 September 1883.



inherited in a more conventional manner, may behave similarly.

Telomeres apparently do not have to contain C-A repeats in all species. Minichromosome termini in the vaccinia virus²³ and in the rDNA of two slime mould species^{24,25} have more complex terminal repetitive blocks which are not based on the C-A dinucleotide. However, the evidence from ciliates and yeast suggests that C-A-containing tracts are widely used in telomeres, where they are substrates both for nicking and for amplification. So perhaps they become dispersed elsewhere as a by-product of these processes.

The lability of (CA)_n blocks is further attested by their association with sites of recombinational events. In man, substantial (CA)_n blocks appear to mark boundaries of gene conversion events both between globin genes⁴ and between immunoglobulin C_κ genes²⁶. Also, a (CA)₄ block marks the boundary of an α -globin gene duplication⁵, and Rous sarcoma virus picked up its *src* gene by recombination within (CAGA)_n repeats²⁷. All this suggests that these blocks are cross-over hotspots, and Stringer²⁸ found a smoking gun: an insertion of simian virus 40 into the rat genome which involved recombination between (CA)_n blocks.

Curiously, telomeres and unequal cross-overs represent only two of many situations in which cleavage of DNA or RNA occurs in the vicinity of a C-A dinucleotide and is followed by ligation or amplification of the free end. Another is the insertion of retroposons (particularly *Alu* sequences

and processed pseudogenes), which involves the generation of a 3' A-rich tail which, when not pure poly(A), is most commonly (CA)_xy. Four specific processes have been described which also usually involve C-A at a DNA cleavage site (where ' ' and ' ' will be used to mark the sites of cleavage on the written and complementary strands respectively). First, almost all eukaryotic transposons end in C-A', and a nick must be made here to permit transposition. Second, the consensus recognition sequence for immunoglobulin V-D-J joining is CACAGTG. Third, the cleavage site for inversion of the halves of the herpes simplex virus genome²⁹ is G,C'A. And fourth, the cleavage site for initiation of yeast mating-type conversion³⁰ is C,AACA'.

In RNA, the best known case is polyadenylation, for which the most common signal is AATAAA. . . .CA; the poly(A) is apparently added following cleavage at

the C-A dinucleotide. A second case is that of the histone mRNAs³¹, whose 3' ends appear by termination or cleavage in or immediately after the sequence CACCA. Third, when influenza virus hijacks capped oligonucleotides from host nuclear RNA³², the preferred cleavage point is immediately after C-A. These three cases may all involve cleavage of a nascent RNA; post-transcriptional RNA cleavages do not favour C-A.

These cases include most of the specific cleavage-ligation and cleavage-polymerization processes which have been precisely characterized in eukaryotes. Certainly they involve a variety of mechanisms, but the common occurrence of the C-A dinucleotide does suggest that the mechanisms may have more similarity than meets the eye. □

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Materials science

Cubic diamonds

from J. Wilks

IN a recent issue of *Physical Review Letters*, M.T. Yin and M.L. Cohen¹ pose the question of whether diamonds will transform under pressure; their answer is that at around 23 Mbar diamond will adopt a crystallographic structure which is simply cubic. This result is of practical importance because of the widespread use of diamonds as anvils in equipment used to generate high pressures. Diamonds are very strong and are available in shapes which permit a pressure applied on a relatively large face to produce a much greater pressure over an opposite face of smaller area. Diamonds are also transparent to a spectrum of electromagnetic radiation, so it is possible to carry out many experiments on materials confined under pressure between the anvils. During the past ten years a wide range of important results on the behaviour of matter at high pressure has been obtained in this way².

At present, measurements are being made quite commonly at pressures up to 500 kbar (~ 500,000 kg cm⁻²). One of the most difficult tasks in this type of work is to obtain accurate values for the pressure within the high-pressure cell, but pressure scales are now reasonably well established up to 500 kbar. Progress to higher pressures involves the steady extension of present techniques, and also the requirement that the diamond anvils will not transform or fail under pressure. Extrapolations from the behaviour of germanium and silicon under pressure made by Van Vechten in 1971 predicted that the structure of diamond would change to the β -tin structure at a pressure of 1.3 Mbar (ref. 3). However, Mao and Bell⁴ subsequently claimed to have reached a static pressure of

1.7 Mbar. They observed evidence of plastic flow in one of the anvils, presumably because of off-centre forces, but no sign of any transition.

The letter by Yin and Cohen examines the possibility of a transition in diamond in a more fundamental way on the basis of an *ab initio* calculation they had previously made to account for the structural properties of silicon and germanium⁵. In this earlier paper, Yin and Cohen assumed only the atomic number of the element and the type of crystal structure and then determined the lattice constants, cohesive energies and bulk moduli at normal pressure by using a pseudopotential theory and local density-functional formalism. Their calculation gave good results for both silicon and germanium at normal pressure, and by examining the behaviour of other possible crystal structures they were also able to account for the transitions to the tetragonal β -tin form observed in silicon and germanium at pressures of about 125 and 100 kbar respectively. Making similar calculations for diamond they predict a transition not to the β -tin form but to simple cubic at a pressure of about 23 Mbar. A transition in diamond may thus ultimately limit the use of diamond anvils, but at 23 Mbar, there is still a long way to go from currently obtainable pressures. □

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Laser-induced chemistry

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Some 20 years ago the invention of lasers led to suggestions that the high-intensity monochromatic light that they produce might be used to bring about a new class of chemical interaction. Over the past 10 years this new chemistry has come of age.

THE introduction of any type of energy into matter can be used to stimulate chemical reactions. For example, the stimulation of chemical reactions by means of thermal energy (thermochemistry), light energy (photochemistry), ionizing radiation energy (radiation chemistry), and electric energy (plasma-chemistry), has found many applications. The laser, as a source of high-intensity monochromatic light, was invented in 1960, and chemistry was recognized as one possible field of application¹. Only now, 20 years later, have we stepped over the threshold of laser chemistry processes in laboratory and pilot setups.

There were two stages in the development of laser chemistry. During the first (1960–71) potential methods of laser chemistry were discussed in the literature, but only during the second stage (1972–82) did the progress in laser engineering and particularly in the development of powerful IR and UV lasers permit a successful demonstration of the photochemical action of laser light based both on well-known ideas of single-quantum photochemistry^{2,3}, and on new methods of multiple and multiphoton excitation of atoms and molecules^{4,5}. The rapid progress in laser chemistry went hand-in-hand with the development of methods of isotope-selective laser photophysics and photochemistry for isotope separation^{6,7}.

Photoexcitation

The methods of laser chemistry can be classified according to the type of photoexcitation. All methods of single-photon linear photochemistry are based on the excitation of the electronic state of the atom or molecule (single-photon electronic photochemistry) or the vibrational state of the molecule (single-photon vibrational photochemistry) as one photon is absorbed (Fig. 1a, b). The excitation of atomic and molecular states by

visible and UV irradiation of ordinary sources is well known in photochemistry⁸. Laser radiation here turns out to be very useful for two reasons. First, the high spectral brightness of laser light makes it possible to excite any discrete quantum state of an atom or molecule without accidental coincidences between the wavelengths of the intense lines of spontaneous radiation of ordinary sources and the absorption lines. Second, the high spectral brightness of IR lasers permits the excitation by molecular vibration that was suggested when the potential application of lasers in chemistry was first discussed¹. The first experiments in IR vibrational photochemistry, however, were performed⁹ soon after this using a thermal source of IR radiation because there were no intense IR lasers available at that time.

The high intensity of laser radiation has allowed new methods of multiphoton, nonlinear photochemistry to be developed, which have no analogy in conventional photochemistry. These methods are based on absorption of a number of laser photons by one particle (an atom or a molecule). But first, techniques of multistep excitation of atomic and molecular electronic states had to be developed^{10,11} (Fig. 1a, c). These methods were based on the ability of laser light to transfer a considerable fraction of atoms and molecules from the ground state to the excited state when the absorption of photons by excited particles becomes probable.

The development of high-intensity IR lasers made it possible to realize^{12,13} collisionless multiphoton excitation of high-lying vibrational levels of polyatomic molecules by intense IR radiation (10^6 – 10^8 W cm⁻²) as shown in Fig. 1b. (Early work on multiphoton excitation of molecular vibrations is reviewed in refs 5, 14.) Methods of multiphoton-vibrational IR photochemistry developed rapidly after the discovery¹⁵ of isotope-selective photodissociation of molecules in intense IR fields, and they are now used for isotope separation.

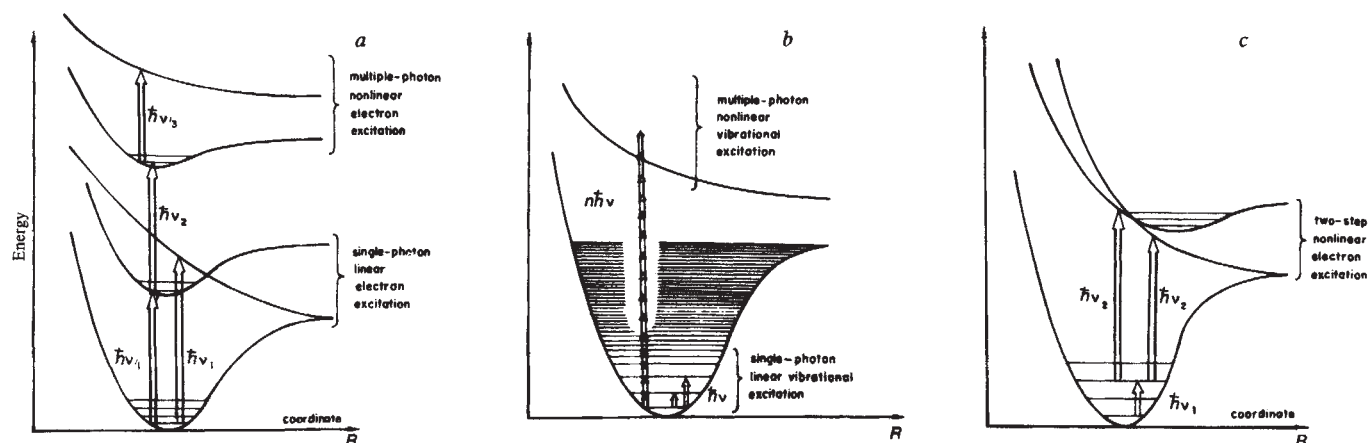


Fig. 1 Various schemes of single-photon (linear) and multiple photon (nonlinear) excitation of vibrational or electronic levels of molecule by laser radiation which form the basis for UV (a), IR (b) and IR-UV (c) photochemistry.

Thus, with modern lasers it is possible to excite the electronic and vibrational levels of molecules. These schemes can be subdivided into electronic (UV) photochemistry with linear and multiphoton excitation (Fig. 1a), vibrational (IR) photochemistry with linear and multiphoton excitation (Fig. 1b), and combined vibrational–electronic (IR–UV) photochemistry with multistep excitation (Fig. 1c). In the first two cases the laser radiation interacts either with the electronic or with the vibrational motion of molecule. Such a subdivision is not strictly due to the inevitable coupling of electronic and vibrational motions. In the final case (Fig. 1c), laser IR and UV radiation excites both the vibrational and the electronic motions of molecules.

Single-photon photochemistry

The unique combination of valuable properties (monochromaticity and tuning of wavelength, high intensity and controllable pulse duration, good directivity and controllable polarization) in laser light sources opens interesting possibilities in the field of photochemistry.

The excellent monochromaticity and the high intensity of laser light make possible the selective excitation of chosen quantum states of atoms and molecules even through very narrow absorption lines at the quantum transition between strictly defined initial and final quantum states. This facilitates the study of the dynamics of chemical reactions of reagents in known quantum states with products also formed in definite quantum states (state-to-state reaction dynamics)^{16,17}. Moreover, the high rate of stimulated transitions makes it possible to excite a considerable fraction of particles (atoms or molecules) to a certain excited state. This allows experiments to be performed at a rather low density of particles in the absence of collisional relaxation, for example, in intersecting molecular beams. The dynamics of the reaction between laser-excited atoms of Sr^* ($^3\text{P}_1$) and unexcited molecules of HCl ($v=0$) are described in ref. 18. Such reactions result in the formation of vibrational distribution ($v' \approx 27-31$).

Selective laser excitation of certain quantum states is particularly useful in studying the dynamics of chemical reactions of excited molecules by enabling the numerous pathways to be traced. This process can be illustrated by a detailed study of the monomolecular reaction of H_2CO photopredissociation¹⁹ or into the bimolecular reaction of excited ICl molecules with acetylene^{20,21}. In both cases a tunable laser excited the chosen electronic–vibrational–rotational states of the molecules.

The high intensity of laser light enables us to interfere with a chemical reaction by exciting the 'transient complex'²² formed during a brief moment of reactant collision. The first experiment of this kind was carried out with intersecting beams of K atoms and BrHgBr molecules²³. The intersection volume of these beams was irradiated by a pulsed dye laser at the 595 nm wavelength which was inconsistent with the absorption lines of both the reactants and the products. As a result, weak luminescence was seen at $\lambda = 500$ nm which was attributed to the emission of electron-excited $\text{HgBr}^*(\text{B})$ molecules. On the basis of this observation it is concluded that light is absorbed by the collisional complex $[\text{KBrHgBr}]$ which dissociates to an excited state yielding KBr and $\text{HgBr}^*(\text{B})$ molecules with the radiative transition $\text{HgBr}^*(\text{B}) \rightarrow \text{HgBr}(\text{x}) + h\nu$ (500 nm).

High spatial coherence and hence the ability of laser light to be focused onto an area with dimensions of the order of the radiation wavelength proved useful for developing methods of laser microphotochemistry for application in solid state electronics²⁴. A focused UV laser beam was used to initiate chemical processes in the micrometre region on the surface of a solid. These processes can be initiated by the photodissociation of molecular gas (metal–organic molecules) in the vicinity of the gas–solid interface. The resulting fragments of the molecules can either react with the surface or desorb on it. Thus either microetching of methyl halide molecules or microdeposition of methyl alkyl molecules is likely to occur on the surface. When

the power of the UV irradiation at $\lambda = 257$ nm is just 3 mW, it is possible to obtain a satisfactory reaction rate from microchemical processes.

Conventional electron photochemistry utilizes UV irradiation. Recently developed efficient UV lasers, particularly excimer lasers²⁵, have provided new possibilities for preparative photochemistry, for example, more effective laser production of vitamin D has been demonstrated²⁶. Another interesting possibility is the production of radicals through photodissociation of molecules using the UV laser which has high 'concentration' and so allows control of the course of chain reactions in a high-pressure gas reactor²⁷. In this case, the concentration of radicals responsible for chain generation can be controlled independently of temperature. This permits, for instance, the synthesis of vinyl chloride in the usual way at a higher yield without generating carcinogenic impurities²⁷. To carry out such a process on an industrial scale, high-power UV excimer lasers with an average power of up to 1,000 W would have to be developed.

The development of IR lasers, particularly those affecting vibrational–rotational transitions of molecules, has provided researchers with a means of exciting the vibrational levels of molecules and studying systematically the reactions of vibrationally-excited molecules. This field of single-photon vibrational photochemistry has developed much less rapidly than electronic photochemistry. Now consideration is being directed mainly towards the effect of increasing the chemical reaction rate as opposed to the thermal reaction rate of molecules using laser excitation of a certain vibrational level. For example, it has been shown²⁸ that a vibrationally-excited molecule of HCl ($v=1$) reacts with an atom of Cl at a rate 10^3 times higher than the HCl molecule at 298 K. Similar experiments have been performed with many other molecules. Early studies into the stimulation of chemical reactions of molecules (in the early 1970s), had given contradictory results, especially when molecular gases have also been excited at pressures of tens of torr and over with the IR cw laser radiation. Many authors had ignored the effect of fast transfer of vibrational excitation to translational degrees of freedom that resulted in bulk heating of the gas without essential heating of the walls of the reactor. (Such experiments are reviewed in refs 4, 29.)

The possibility of bond-selective photochemical reactions of polyatomic molecules is an interesting aspect of laser vibrational photochemistry³⁰. Is it possible to control the chemical reaction of an excited molecule if energy is expended not on all the vibrational degrees of freedom but on one definite vibration, a definite molecular bond? This is possible, in principle, if the rate of vibrational energy randomization is lower than that of the chemical reaction. This must be observed when exciting vibrations slightly coupled to the rest of vibrations as, for example, in exciting the harmonics of the CH -bond of a polyatomic molecule such as allyl isocyanide ($\text{H}_2\text{C}=\text{CHCH}_2\text{NC}$) in experiments³¹.

Thus, in contrast to single-photon electron photochemistry, single-photon vibrational photochemistry is still in the fundamental study stage, a long way from being applied in practice.

Multiphoton vibrational photochemistry

The intense IR radiation of a pulsed laser at a frequency tuned to the vibrational absorption band of any polyatomic molecule induces very fast vibrational excitation of that molecule. In other words, under a resonant IR field the vibrational degrees of freedom are subjected to strong heating which depends on the number of IR photons absorbed by the molecule. Under the action of an IR pulse with its energy fluence varying from 1 to 10 J cm^{-2} it is relatively easy to deposit energy of 1–10 eV into a polyatomic molecule via the absorption of, say, 10–100 photons from a CO_2 laser operating in the region of $10 \mu\text{m}$. A highly vibrationally-excited molecule can undergo, first, various monomolecular reactions (such as dis-

sociation, fragmentation and isomerization), and then the decomposition products of a polyatomic molecule, such as active radicals, can themselves participate in subsequent reactions. All this has particular interest for chemistry and, in essence, has given birth to a rapidly developing trend in photochemistry, IR multiphoton (MP) photochemistry (see review in refs 4, 32–34, Chapter 5 in ref. 5).

The interest shown in this new trend in photochemistry can be explained by the following characteristics of multiphoton excitation of vibrations: (1) laser excitation is purely radiative and does not require collisions; (2) laser excitation can be very rapid due to the high rate of laser energy deposition into a molecule that may be as high as 10^{12} eV s⁻¹ in the case of powerful picosecond IR lasers; (3) laser excitation is resonant since it can be selectively realized for a certain type of polyatomic molecule in gas mixtures. Each of these properties provides new possibilities for chemists.

The collisionless character of multiphoton excitation ensures the vibrational excitation of molecules without any heating of the translational degrees of freedom. This enables detailed studies to be made into the rate of monomolecular reactions of highly excited polyatomic molecules in isolated conditions and makes it possible to correlate the results of experiments with predictions of the statistical RRKM theory³⁵. Some laboratories have performed experiments of this kind using a variety of techniques: molecular beams³³, picosecond IR pulses³² and by measuring the fraction of molecules interacting with the IR field and the value of energy absorbed by them³⁶. In all these experiments the predictions of the RRKM theory are in good agreement with the results obtained from measuring the rate and yield of the multiphoton IR photodissociation. This means that all the vibrational modes are involved in the process of multiphoton excitation, or, in other words, vibrational excitation is randomized in the experimental conditions. This has been also proved by the results of direct Raman probing of the excitation level of one vibrational mode during multiphoton excitation with a transfer rate of 10^7 s⁻¹ to the other vibrational mode³⁷. The above-mentioned studies into multiphoton IR photophysics and photochemistry of polyatomic molecules were carried out mainly in physical laboratories and published in physical journals. Now the focus of this research has moved into chemical laboratories and publications can be found in chemical journals (see, for example, refs 38–41).

Collisionless multiphoton IR excitation has made it possible to study monomolecular reactions of short-lived molecules (radicals, molecular ions) which cannot be excited by collision because they lose their excess energy in collisions. This technique was applied to the study of the dissociation of the CF₃O radical⁴² and various molecular ions^{43,44}.

A high rate of multiphoton excitation makes the process of IR dissociation of polyatomic molecules unstable since it admits strong vibrational excitation of a molecule over the limit of its dissociation. It is known that, if the vibrational energy E , of a molecule exceeds the dissociation energy D , the molecule is capable of spontaneous decomposition to fragments. The monomolecular decomposition rate $K(E)$ in a simplest approximation is described by the expression³⁵:

$$K(E) = \mathcal{K} \left(\frac{E - D - E_0^\Delta}{E + E_0^\Delta} \right)^{s-1} \quad (1)$$

where s is the number of vibrational degrees of freedom; E_0^Δ and E_0^Δ are the zero-point energies of molecule and activated complex respectively; $\mathcal{K}$ is the frequency factor equal in order of magnitude to the frequency of molecular vibrations (10^{12} – 10^{14} s⁻¹).

It is obvious that the decomposition rate imposes a limitation on the maximum possible value of molecular excitation ($E_{\max} - D$) over the dissociation limit at a given rate of multiphoton excitation. The IR laser pulse intensity $I = 10^7$ – 10^8 W cm⁻² and the vibrational absorption cross-section $\sigma_{\text{abs}} = 10^{-19}$ cm² being

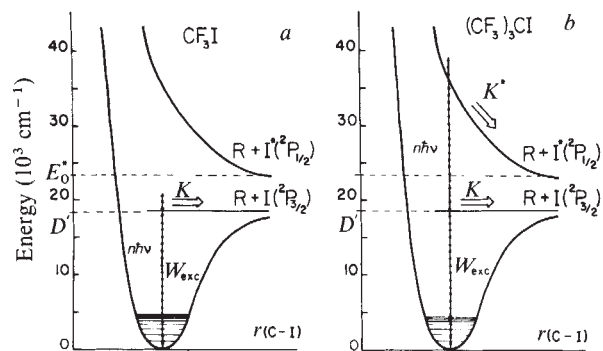


Fig. 2 Vibrational multiphoton excitation over the dissociation limit under intense IR laser pulses is small for the CF₃I molecule (a) and very large for a more complex molecule such as (CF₃)₃CI (b) which leads to electronic predissociation of this molecule⁴⁶.

typical, it is easy to reach the multiphoton excitation rate $W_{\text{exc}} = \sigma_{\text{abs}} I = 10^8$ – 10^9 s⁻¹. The monomolecular decomposition rate $K(E)$ increases drastically with increasing $(E - D)$ but the rate of such an increase drops as the number of vibrational degrees of freedom s is increased, that is as the number of atoms in the molecule is increased. The maximum vibrational overexcitation of a polyatomic molecule in the ground electronic state can be estimated from the simple relationship $W_{\text{exc}} \approx K(E_{\max})$.

Now compare the value of $(E_{\max} - D)$ for two different molecules, CF₃I and (CF₃)₃CI. In the case of CF₃I ($s = 9$) this estimation gives $E_{\max} - D \approx 0.1D$, so that the overexcitation is not large ($\sim 2,000$ cm⁻¹), as has been shown in experiments with molecular beams⁴⁵. At the same time, for the molecule (CF₃)₃CI ($s = 36$), $E_{\max} \approx 2D$ and the molecule can be considerably overexcited before its dissociation begins.

This conclusion is supported by experiments⁴⁶ in which the electronic predissociation of the vibrationally-overexcited molecule (CF₃)₃CI was observed (Fig. 2). Indeed, at such strong overexcitation the vibrational energy of the (CF₃)₃CI molecule, $E \approx 40,000$ cm⁻¹, exceeds the energy limit of its first electron term ($E_0^* = 28,000$ cm⁻¹) that is unstable. A nonadiabatic coupling of the electronic and vibrational motions of the excited molecule results in its electronic predissociation into electron-excited $I^*(^2P_{1/2})$ atoms the formation of which can be observed from luminescence at $\lambda = 1.315$ μm (ref. 46). At the same time, the IR multiphoton dissociation of the CF₃I molecule, because of limited vibrational overexcitation, gives birth only to I atoms in the ground state ($^2P_{3/2}$).

Thus, by increasing the multiphoton excitation rate W_{exc} one can reach very high vibrational levels of a molecule lying in the region of its real continuum and hence examine new modes of dissociation of a polyatomic molecule. This technique has made it possible in experiments with the anthracene molecule ($s = 66$) for multiphoton excitation of CO₂ lasers to impart the vibrational energy $E = 16$ – 17 eV to the molecule and to observe new dissociation channels with electron and proton detachment⁴⁷.

Powerful picosecond IR pulses provide very high rates of multiphoton vibrational excitation of molecule $W_{\text{exc}} = 10^{12}$ – 10^{13} s⁻¹. In these conditions it will probably be possible to study experimentally such fundamental questions as vibrational energy randomization and to observe the changeover from random vibrational motion to a regular motion where vibrational energy is concentrated in a small number of modes⁴⁸. In other words, the conditions of experiments (picosecond IR pulses and large molecules), in which mode-selective multiphoton excitation is possible, cannot yet be realized.

The resonant character of multiphoton IR excitation makes possible the selective excitation and dissociation of a polyatomic molecule of chosen type in a gas mixture. Such intermolecular selectivity is fundamental for the application of this method in

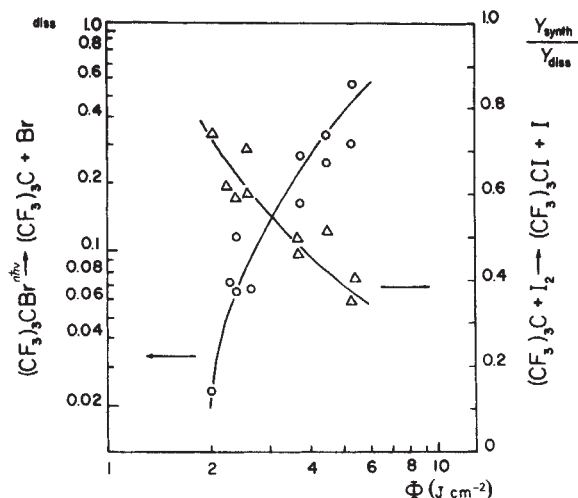


Fig. 3 Dependence of the dissociation yield Y_{diss} of the $(\text{CF}_3)_3\text{CBr}$ under the action of CO_2 laser pulses with the energy fluence Φ (left scale) and the corresponding dependence of the ratio of the synthesis yield Y_{synth} of $(\text{CF}_3)_3\text{CI}$ to the dissociation yield of $(\text{CF}_3)_3\text{CBr}$ (ref. 50).

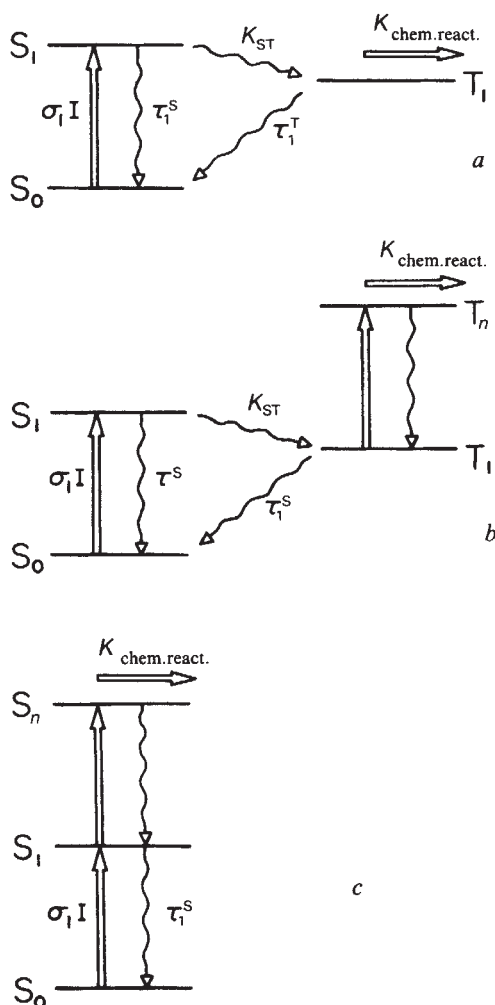


Fig. 4 Excitation schemes of the electronic states of polyatomic molecules and the corresponding channels of photochemical reaction: *a*, single-photon excitation of the S_1 singlet state with subsequent transition to the T_1 triplet state; *b*, two-step excitation via the intermediate triplet state T_1 under the action of short laser pulses of moderate power; *c*, two-step excitation via an intermediate singlet state under the action of high-power ultrashort pulses.

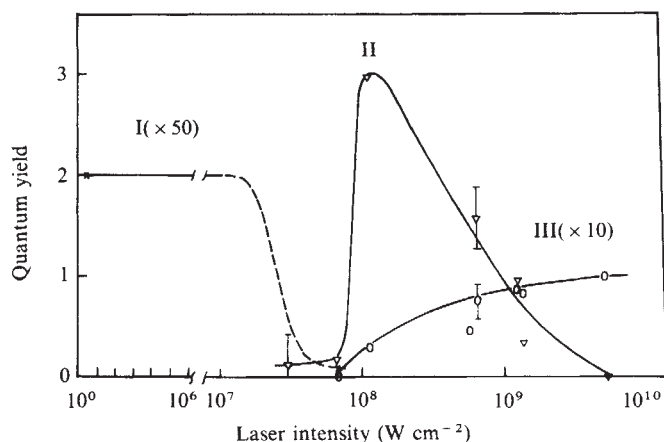


Fig. 5 Dependence of the quantum yield of photoproducts formed from irradiating maleic acid in aqueous solution on UV intensity: I, the formation of fumaric acid due to *cis-trans* isomerization of maleic acid at low intensity; II, the formation of maleic acid dimers due to radical dimerization under irradiation with nanosecond pulses of moderate intensity; III, the formation of malic acid due to water addition to the $\text{C}=\text{C}$ bond of maleic acid under irradiation with picosecond pulses of high intensity⁶⁵.

laser isotope separation⁵. This problem has been considered comprehensively elsewhere⁴⁻⁷ and here we are going to consider the application of this method in chemistry.

It is of interest to the chemist to produce radicals of one definite sort by multiphoton dissociation of polyatomic molecules. This method can be used to produce radicals at high concentrations without heating the gas mixture, thus making it possible to initiate and strictly control biomolecular radical reactions in the gas phase that essentially differ from thermal initiation of gas-phase radical reactions of polyatomic molecules. Under thermal initiation many final products are usually formed and the yield of the desired product is low. This is conditioned by a large number of potential chemical reactions in a multicomponent mixture when under thermodynamic equilibrium the initial, intermediate and final products have the same high temperature. New possibilities for high-efficiency gas-phase radical synthesis of molecular compounds at IR multiphoton excitation and dissociation of one of initial molecular compounds have been demonstrated elsewhere^{49,50}. Bagratashvili *et al.*⁵⁰, for example, observed effective synthesis of the $(\text{CF}_3)_3\text{CI}$ molecule during IR multiphoton photodissociation of $(\text{CF}_3)_3\text{CBr}$ molecules surrounded by I_2 molecules. In this experiment controllable synthesis of $(\text{CF}_3)_3\text{CI}$ with a high yield (up to 60%) and the suppression of the formation of other products typical of thermal reactions (Fig. 3) were obtained. This is to be expected since in IR photochemical decomposition of initial molecules (as well as in UV photochemistry) the resulting products, radicals $((\text{CF}_3)_3\text{C}$ in the experiment⁵⁰), have comparatively low energy, and, as they slightly interact with IR radiation, they are not subjected to further decomposition. At the same time, in thermal conditions the resulting polyatomic radicals inevitably undergo further decomposition since the energy of their dissociation is usually lower than the dissociation energy of the initial molecule. Analysis⁵¹ shows that, in suitable experimental conditions, IR multiphoton photodissociation of definite molecules in gas mixtures can produce one final product with a yield of almost 100%.

Multiphoton electronic photochemistry

As in the case of powerful IR radiation, intense visible or UV radiation induces multiphoton excitation of polyatomic molecules but through the electronic intermediate resonant levels. To deposit energy of ~ 10 eV, for example, a molecule must absorb just 2 or 3 UV photons. Multiphoton excitation of molecules induces various photochemical processes; photo-

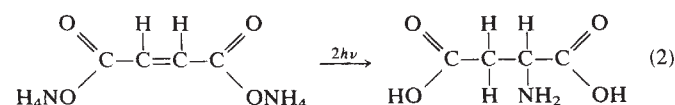
fragmentation and photoionization by way of high-lying electronic states, production of active radicals which react with the environment (with the molecules of the solution, for example). The photophysics and photochemistry of molecules in highly excited electronic states are only now beginning to be studied comprehensively. Multiphoton ionization of polyatomic molecules in combination with mass-spectrometric analysis of contaminated fragments is becoming an important analytical method⁵² (see reviews in refs 53–55). Another approach, multiphoton excitation of organic molecules in solutions, is of interest for preparative photochemistry and photobiochemistry. Let us consider briefly these possibilities which have been demonstrated successfully in recent experiments.

Most photochemical reactions of molecules in solutions are based on exciting the singlet state of the molecules and their subsequent transition to a long-lived triplet state (Fig. 4a)⁸. The molecules in a long-lived triplet state can absorb another photon and transmit to a high-lying triplet state⁵⁶ (Fig. 4b). Such successive absorption of two photons can be observed with intensity of light $I_{ST} = \hbar\nu/\sigma_1\tau_1^T$, where σ_1 is the cross-section of the electron transition $S_0 \rightarrow S_1$, τ_1^T is the triplet state lifetime. At typical values $\sigma_1 = 10^{-17} \text{ cm}^2$ and $\tau_1^T = 10^{-4} - 10^{-7} \text{ s}$ the intensity should be $I_{ST} = 10^3 - 10^6 \text{ W cm}^{-2}$ that can be easily obtained with the use of UV lasers generating pulses with $\tau_p \approx 10^{-8} \text{ s}$. The energy fluence of such a pulse $\phi = I_{ST}\tau_p \approx 10^{-2} \text{ J cm}^{-2}$ is much lower than the damage threshold of a condensed medium by a laser pulse and hence such two-step excitation is attainable.

Under the action of a more powerful laser pulse of intensity $I_{SS} = \hbar\nu/\sigma_1\tau_1^S$, where τ_1^S is the excited singlet state lifetime, the probability of absorption of another photon at the next singlet transition $S_1 \rightarrow S_n$ can become larger than the probability of transition to a triplet state (Fig. 4c). Because of a very short lifetime $\tau_1^S = 10^{-9} - 10^{-11}$, the required laser pulse intensity will $I_{SS} \approx 10^9 - 10^{10} \text{ W cm}^{-2}$. Such intensities are attainable using powerful lasers generating nanosecond and picosecond pulses. However, in practice it is impossible to apply nanosecond pulses to such two-step excitation of high-lying singlet states since the energy fluence of such a pulse, $\phi = \tau_p I_{SS} \approx 1 - 10^2 \text{ J cm}^{-2}$, considerably exceeds the nonlinear damage threshold of a condensed medium by powerful laser radiation. So one should use powerful picosecond pulses to realize photochemical processes through high-lying singlet states. Pulses of duration $\tau_p \approx \tau_1^S$ are optimal for realizing a high efficiency of two-step excitation.

Photochemical processes induced by two-step laser excitation of high-lying electronic states have been demonstrated for many organic molecules in solution, and particularly photodecomposition of nucleic acid bases in aqueous solution under the action of picosecond UV (265 nm) pulses through an intermediate singlet state^{57,58}, photodecomposition of porphyrin molecules in aqueous solution⁵⁹, photodissociation of dissolved benzene derivatives⁶⁰, and photodecomposition of a complex of porphyrin with dye molecules⁶¹. Note that the process of two-photon excitation of molecules via virtual states has been used successfully⁶² to excite molecular states with energies $E = 6 - 8 \text{ eV}$ which in case of single-photon excitation can be populated only with the use of a vacuum-UV source. In many cases single-photon excitation by such short-wave radiation is almost impossible because of its strong absorption in solvents.

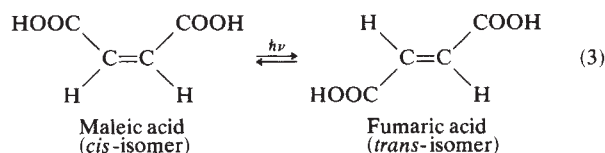
Two-step excitation of molecules through real singlet or triplet electron states (Fig. 4b, c) has been successfully used to stimulate photochemical synthesis of organic molecules in solutions via high-lying electron states. For example, it has been found⁶³ that, as the aqueous solution of ammonium salt of maleic acid is irradiated by powerful UV (265 nm) picosecond radiation, ammonia adds to the double bond of acid —C=C— yielding the corresponding α -amino acid



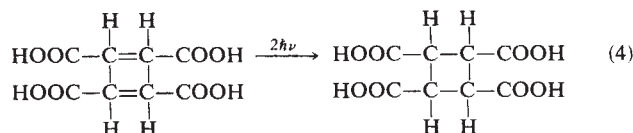
The yield of such a photochemical reaction stimulated by two-step excitation of molecules through intermediate singlet state may be as high as 100%.

Under the action of UV nanosecond pulses, carvone to carvone camphor photocyclization due to two-step excitation through intermediate triplet state was observed⁶⁴.

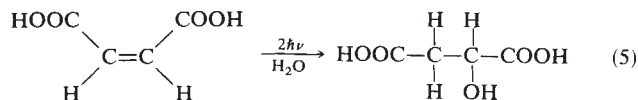
In experiments on nonlinear UV photochemistry of maleic acid in aqueous solution it has been shown⁶⁵ that, as the UV laser radiation intensity increases, the channels of photochemical reactions change. Comprehensive studies into the photochemistry of maleic acid under low-intensity UV light have been carried out⁶⁶. In this case, in aqueous solution, maleic acid is isomerized through excitation of a triplet state (region I, Fig. 5):



Under the action of nanosecond UV pulses (265 nm) photodimerization can be observed which occurs due to two-step excitation via triplet states to the region of intensity from 3×10^7 to 10^9 W cm^{-2} (region II, Fig. 5). Dimers are formed due to the breaking of the double bond —C=C—



Under the action of picosecond UV pulses (265 nm) with their intensity ranging from 10^8 to $6 \times 10^9 \text{ W cm}^{-2}$ a reaction of water addition due to excitation of singlet molecular state takes place (region III, Fig. 5)



Thus, by changing only one radiation parameter, intensity, it is possible to stimulate qualitatively-different types of photochemical transformations and selectively to produce photochemical products with a high quantum yield.

Photochemical transformations of molecules with multiple —C=C— bonds under the action of high-power UV picosecond laser pulses are worthy of detailed study since, first, olefins are one of the most abundant classes of chemical compounds and, second, photochemical reactions with double-bond breakage open the way for the photochemical synthesis of many compounds which cannot be synthesized by methods of single-photon linear photochemistry.

In conclusion it should be noted that photochemical transformations of biomolecules stimulated by laser picosecond radiation open a wide range of applications for photobiochemistry and will make it possible to develop laser pharmacology when the molecules required are decomposed or synthesized *in vivo* and *in situ*.

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ARTICLES

Sea ice thickness distribution in Fram Strait

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HM nuclear submarine Sovereign has obtained the first synoptic measurements of the ice thickness distribution in Fram Strait, the main outflow from the Arctic Ocean. The ice nearest the ice edge is of quite different character from ice in the interior of the Strait, with a greater proportion of first-year ice and less pressure ridging. An estimate of the ice flux through Fram Strait can be made from the distribution of mean ice drafts.

FRAM STRAIT, the deep passage between Svalbard (Spitsbergen) and Greenland, is of great importance to the climatology of the Arctic, because it handles 90% of the heat exchange and 75% of the mass exchange between the Arctic Ocean and the rest of the world ocean¹. The East Greenland Current which flows down its western side is a continuation of the Trans Polar Drift Stream and is the chief medium for ice export from the Arctic Ocean, while the east side of the Strait is kept open by the West Spitsbergen Current, a warm offshoot of the North Atlantic Current. North of Svalbard, where this current sinks beneath less dense polar surface water, it keeps open a bight in the ice edge known as Whalers' Bay or Svalbardbukta² which is the northernmost year-round ice-free sea area in the world. Fram Strait is the location for a major international experiment, MIZEX (Marginal Ice Zone Experiment), to take place from 1983 onwards, involving the use of ships and aircraft to study energy exchanges at the ice margin^{3,4}.

While the ice velocity in Fram Strait has been studied using satellite-tracked buoys^{5,6} and sequential Landsat images⁷, the ice thickness distribution across the Strait was unknown and therefore valid mass flux estimates could not be made. The submarine-borne upward-looking echo sounder is the only

means of making synoptic ice draft measurements, as no air-borne technique has yet been developed to sound sea ice. In April–May 1979, HM nuclear submarine *Sovereign* undertook a cruise to this region which differed from an earlier voyage in October 1976⁸ both by the season and by the fact that the vessel spent several days in the Fram Strait area instead of proceeding directly into the Arctic Basin. The convoluted track that she followed permitted a dense grid of ice draft distributions to be constructed from her upward-looking echo sounder profiles, sufficient to generate contours of mean ice draft.

Mean ice drafts

The profiles were obtained by an Admiralty pattern 45-kHz sonar of similar beam shape to that used on the 1976 cruise. The chart rolls were subsequently released to Scott Polar Research Institute and processed in the same way as data from the earlier cruise. Details of the processing are given elsewhere⁸; corrections were made for submarine speed and depth variations and for the effect of beamwidth on apparent ice draft. The final product was a corrected ice draft profile in digital form at a uniform horizontal spacing of 1.5 m. The profile was

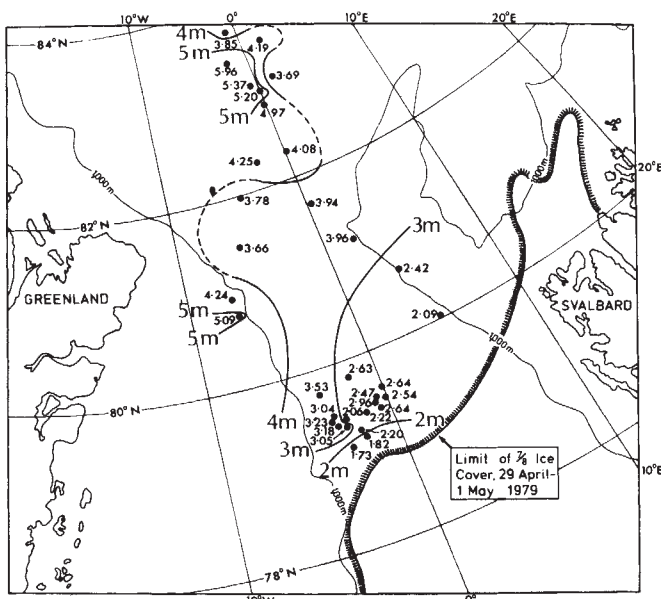


Fig. 1 A contoured map of the mean ice draft in Fram Strait and its approaches 22 April–4 May 1979, constructed from 50-km sections of submarine profile. Drafts are in metres. The 1,000-m ocean depth contour is also shown.

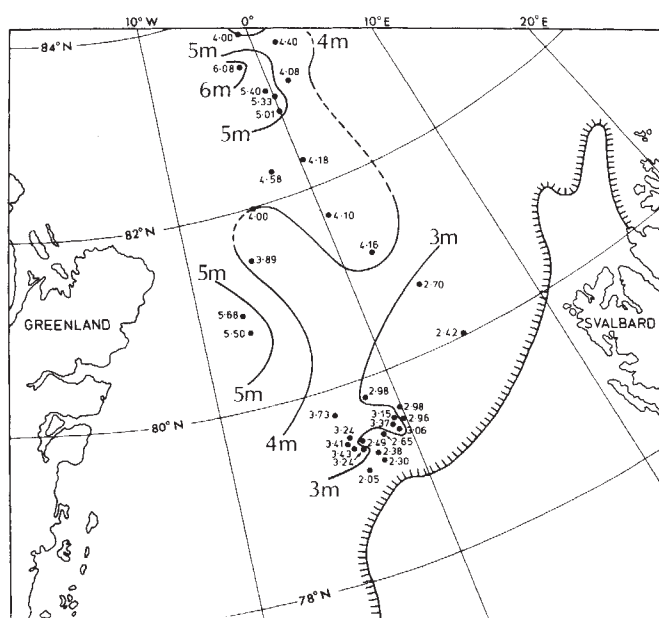


Fig. 2 Modified contours of mean draft after all ice of draft <1 m has been removed from the distributions.

divided into 50-km lengths, and the mean draft in each 50-km section was plotted on a chart at the centroid of the section concerned. The result is shown in Fig. 1. It has been estimated⁹ that the standard error in mean draft over a 50-km interval is ± 0.06 m.

The ice edge position in Fig. 1, which shows a well developed Whalers' Bay at 81° N, 12–19° E, was drawn from an ice chart issued by the Naval Polar Oceanography Center, Suitland, Maryland, representing an analysis of satellite imagery for the period 29 April to 1 May. The sonar recordings themselves were made during 22 April to 4 May, with the Fram Strait portions being done mainly towards the end of that period.

The most striking aspect of Fig. 1 is the rapid decrease in mean ice draft as the ice edge is approached. The variation with distance from the ice edge is most rapid in Fram Strait itself, and is slower to the north-west of Svalbard. Deeper into the Arctic Ocean we notice exceptionally large mean drafts to the north-east of Greenland; these were observed in the earlier cruise⁸ and ascribed¹⁰ to the build-up of pressure ridging as ice in the Trans Polar Drift Stream moves towards the downstream land boundary of Greenland. There is excellent agreement between Fig. 1 and mean drafts observed on the outward and return tracks of the October 1976 cruise⁸, which is evidence of stationarity in the ice draft distribution over this region.

The region near the ice edge, known as the marginal ice zone (MIZ), is known to possess an ice morphology which differs from the interior pack^{10,11}. The ice is broken up into floes by the action of ocean waves which penetrate the ice field to a distance of the order of 100 km (refs 12, 13), the maximum floe size diminishing as the ice edge is approached. As a result of this fracture, and of the free boundary on one side, the ice can respond rapidly to wind forcing. In particular it can easily open up into a diffuse cover under an off-ice wind, permitting rapid growth of new ice in winter in the freshly opened leads and polynyas. Ice-edge eddies can also cause divergence of the pack¹⁴. It is therefore natural to suppose that the low mean ice draft in the MIZ may be due to the interposing of large quantities of young ice among the floes of a formerly compact ice field that was advected into Fram Strait from the Arctic Ocean.

To test this hypothesis we removed all ice of draft <1 m from the distributions and generated new mean drafts for the remain-

ing ice. Thorndike *et al.*¹⁵ estimated that ice growing thermodynamically will reach a draft of 1 m (thickness 1.11 m) in late April after a growth period of 76 days. This is more than sufficient to remove all locally grown ice from the distributions, given the rapid speed of ice drift through Fram Strait¹⁶. Thin ice was found to be more common in the MIZ than in the interior. For sections with mean drafts in Fig. 1 of <3 m, the percentages of ice with <1 m draft ranged from 9.3 to 24.8 (mean 16.6), while in the interior the range was 0.7 to 10.7 (mean 5.4). The only exception was the section closest to the Greenland coast (original mean draft 4.24 m) where 29.4% of the ice was <1 m in draft. This is evidence of the winter coastal polynya that is often seen in this location¹⁰; it appears to have been intersected by the submarine track which generated this section but not the section slightly further to the south.

Figure 2 shows that removal of the thin ice from the distributions produced no significant change in the pattern of variations of mean ice draft. The steepest gradient of ice draft still lies normal to the ice edge and there is still a major difference between the mean ice drafts in the MIZ and in the interior. We must reject the hypothesis, therefore, that the ice in the MIZ has the same character and origin as the interior ice, except for an added thin ice component.

Ice composition

A difference in character between ice regimes can take one or both of the following forms: (1) a difference in ice composition, that is, the relative amounts of first-year ice (ice which has not undergone a summer melt season) and multi-year ice (ice which has undergone one or more melt seasons and become stronger and less saline); (2) a difference in the degree of pressure ridging in the ice field. To distinguish between these possibilities we tested for 'level ice', defined empirically as ice with a local bottom slope of <1 in 40 (refs 8, 17, 18). This analysis picks out mainly ice which is undeformed—has grown thermodynamically from open water—in which case its draft is a direct measure of its age. We divided the experimental area into three regions (Fig. 3): region 1 is the MIZ, where the mean ice draft is <3.5 m; region 2 is the interior pack of Fram Strait and the zone to the north; region 3 comprises those few sections off

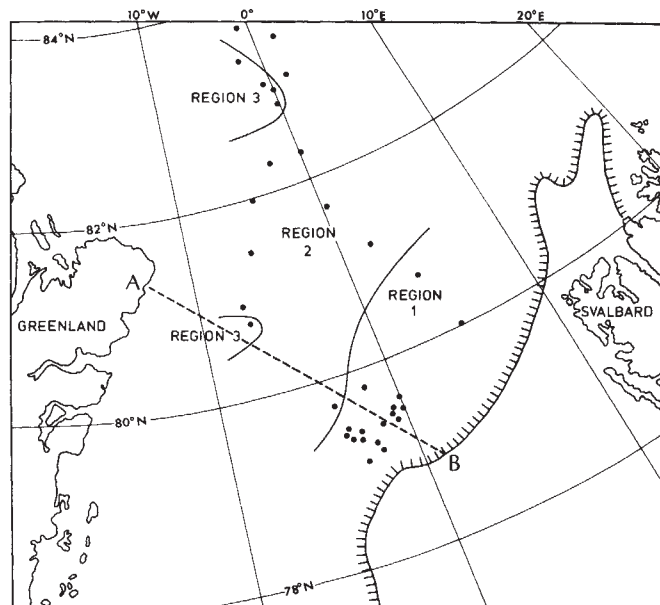


Fig. 3 Division of the experimental area into three regions: region 1 has mean draft of <3.5 m; region 2 covers interior ice; region 3 is heavily ridged zone with mean draft >4.95 m. AB is a cut across Fram Strait for flux estimation.

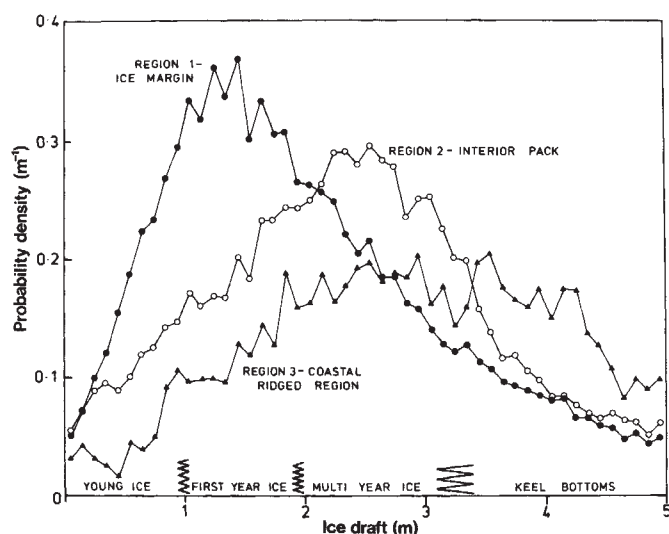


Fig. 4 Probability density function of level ice draft for the three regions defined in Fig. 3.

north-east Greenland where the mean ice draft exceeds 5 m. Figure 4 shows the probability density function of level ice draft for each region. There is a dramatic difference between the MIZ and the interior pack. In region 1 the mode of the distribution lies in the draft range characteristic of first-year ice (<2 m). In region 2 the mode lies in the draft range for multi-year ice (2 m to beyond 3 m (refs 15, 19)). Region 3 follows the general shape of region 2, but with additional level ice at thicknesses beyond 3.5 m which almost certainly consists of pressure ridge bottoms.

We conclude that, during the observation period, ice within 100 km of the ice margin in the Fram Strait was mainly first-year ice, while ice in the interior of the drift stream at latitudes 79 – 84° N was mainly multi-year. The exceptionally thick ice off north-east Greenland differs from the interior ice only by being more heavily ridged. The result strongly suggests a difference in origin. Charts of mean long-term ice drift in the Arctic²⁰ show an eastern ice stream, where ice is collected from north of the Barents and Kara Seas and transported westwards across the north of Svalbard and down into Fram Strait. A

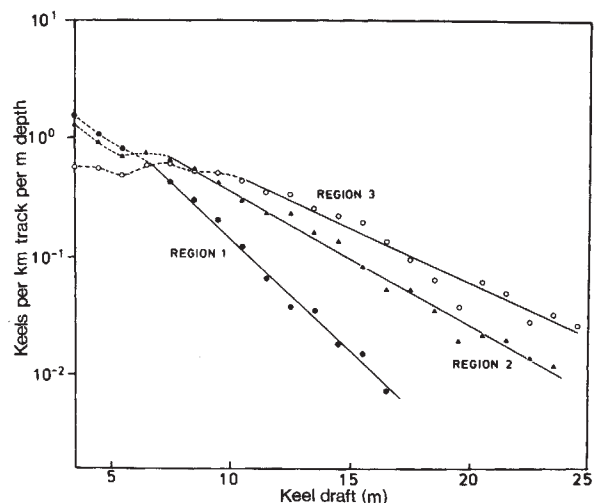


Fig. 5 Distributions of pressure ridge keel drafts for the three regions defined in Fig. 3, plotted on a semilogarithmic scale, with a negative exponential function fitted to the deeper portions of each curve.

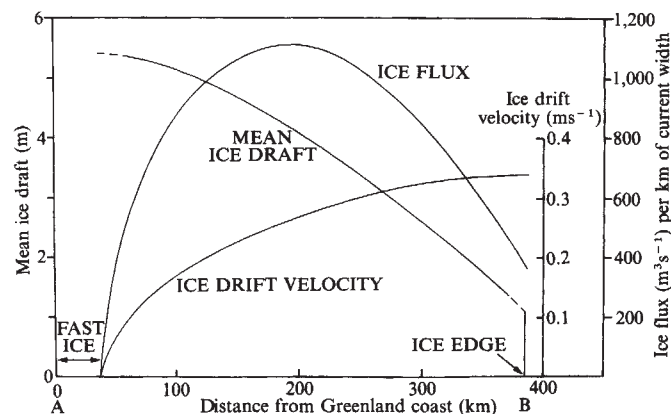


Fig. 6 Ice thicknesses, velocities and mass fluxes across the line AB defined in Fig. 3. The mean ice draft is a smoothed curve derived from the contours of Fig. 1; the ice drift velocity is taken from Vinje¹⁶ for the period April–May 1976; the ice flux is a product of the two. Ice velocity is zero for the first 37 km from the Greenland coast because of landfast ice.

second ice stream, which amalgamates with this, is a transport across the North Pole region from eastern Siberia and which therefore contains a greater proportion of multi-year ice (73%, according to observations during the British Trans Arctic Expedition²¹). Koch²² proposed that in the East Greenland Current the ice in the eastern part of the drift, nearest the ice edge, comes from the eastern ice stream while ice in the western part of the current comes from north and west in the Arctic Ocean. Such a laminar flow concept has seemed unlikely, in view of satellite-tracked buoys which show a high degree of meandering and random motion in the ice drift, ensuring more or less complete mixing of ice types^{10,16}. The present observations revalidate Koch's original idea, at least for the region of Fram Strait itself, that is, the northerly part of the East Greenland Current. We propose, then, that MIZ ice has come westward across the north of Svalbard from the seas north of the Barents and Kara Seas, while the ice in the western part of Fram Strait has come from deep within the Arctic Ocean and has a major multi-year component.

Pressure ridges

We also investigated the other distinguishing ice characteristic, pressure ridging. A pressure ridge is a linear deformation feature produced by the crushing of young ice in a lead, and a submarine profile intersects pressure ridges at unknown crossing angles. An arbitrary criterion must therefore be used to define 'independent ridges' in the profile. The most common definition^{8,17,18,23} is similar to the Rayleigh criterion for resolving spectral lines, and states that a keel (the underwater part of a ridge) is independent if its point of maximum draft is bracketed by points of minimum draft whose depth below the local undeformed ice bottom is less than half that of the crest. Using this definition, analyses were done for regions 1, 2 and 3, and Fig. 5 shows the results. In all cases, at great depths the distribution of keel drafts h follows a negative exponential form

$$n(h) dh = A \exp(-ah) dh \quad (1)$$

where A , a are parameters and $n(h)$ is the number of keels per unit track length and unit draft increment. This form agrees with earlier observations from the Beaufort Sea¹⁸ and M'Clure Strait²⁴. At shallow depths the distributions diverge from equation (1) in the direction of smaller keel numbers. This was explained by Lowry and Wadhams²³ as a shadowing effect due to shallow keels not being resolved when they are very close to deeper keels.

The chief feature of the three distributions, however, is the radical difference in keel frequencies. In region 1 the absolute numbers of keels are lower than in region 2, and also the numbers fall off more rapidly with increasing depth so that there are very few deep keels present in the ice field. To a lesser extent region 2 exhibits the same properties relative to region 3. If we consider keels deeper than 9 m, to eliminate the shadowing effect, we find that the average keel densities are:

Region 1	0.50 keels per km track; mean draft 10.97 m
Region 2	1.81 keels per km track; mean draft 12.69 m
Region 3	2.88 keels per km track; mean draft 13.18 m

Again, the only feasible explanation is that region 1 and region 2 comprise ice of different origins. Ridge building near a lee coast is an observed fact, predicted by models²⁵, and explains the anomalous ridging of region 3. However, ridging in the interior pack does not appear to be a rapid process, as there is no significant difference between the keel frequencies in upstream and downstream sections of region 2. Therefore, it cannot be the case that region 1 represents the baseline ridging level in the Arctic, and that the excess ridging in region 2 has all occurred in Fram Strait. Instead, it is clear that the ridging in region 2 is characteristic of Arctic Ocean interior ice (this also agrees with ridging levels observed deeper in the Arctic in 1976⁸) whereas region 1 ice comes from a closer source and is not old enough to have acquired significant pressure ridging.

Ice flux through Fram Strait

The mean draft contours of Fig. 1 enable us to make an estimate of ice mass transport through Fram Strait. Unfortunately, at

the time of the observations no independent measurement of ice velocity was available, because none of the satellite-tracked buoys of the Arctic Ocean Buoy Program⁵ was in the vicinity. However, a distribution of ice velocities across Fram Strait was obtained by Vinje¹⁶ from Landsat imagery for 21 April–7 May 1976, at a time when the surface pressure field in the Greenland Sea resembled that occurring during the same period in 1979⁵, that is, leading to a strong northerly wind ($5\text{--}15\text{ ms}^{-1}$) for most of the time. We drew a line AB across Fram Strait as shown in Fig. 3 and plotted the distribution of mean ice draft along it from the contours of Fig. 1, with additional examination of *Sovereign* profile sections to allow extrapolation to the ice margin and to the Greenland fast ice edge. We plotted mean ice drift speed normal to AB from the contouring of Vinje¹⁶ for April–May 1976, and thus obtained the distribution of ice flux as a product of these two curves. Figure 6 shows the results. The ice mass transport is greatest in the centre of the drift stream, some 200 km from the Greenland coast and from the ice edge, because although the current is greatest at the ice margin the mean ice draft is lowest there.

Integrating the ice flux across the whole Strait gives us a value of $0.29 \times 10^6\text{ m}^3\text{ s}^{-1}$ of water equivalent for the ice transport through Fram Strait, that is, 0.29 Sv in oceanographic terminology. This exceeds earlier estimates such as that of 0.2 Sv from Vinje⁷ for the same ice velocity profile. Note that this is a short-term average for a period of northerly winds.

Vinje, like most authors, took 3 m as his estimate for the mean thickness of the ice passing through the Strait. An average across the Strait computed from Fig. 6 gives 3.64 m as the mean draft and thus 4.06 m as the mean thickness. This implies that previous estimates of annual ice flux must be revised upwards, which is important because the heat flow associated with the ice transport (estimated at $8 \times 10^9\text{ kcal s}^{-1}$)¹ comprises ~30% of the total heat budget of the Arctic Ocean.

Earlier annual ice flux estimates were based on estimates of the area of ice outflow through the Strait per year. Vinje¹⁶ estimated 1.32, 1.08 and $0.75 \times 10^6\text{ km}^2\text{ yr}^{-1}$ as upper, mean and lower limits, respectively, based on the varying width of the ice stream. Volkov and Gudkovic²⁶ estimated $0.9 \times 10^6\text{ km}^2$ by indirect methods, while Hibler²⁵ obtained $1.3 \times 10^6\text{ km}^2$ using a numerical model run with a single year's pressure data. We may take $0.9\text{--}1.3 \times 10^6\text{ km}^2$ as the likely range of the average area (the year-to-year variability is unknown but is probably consistent with the extreme range of Vinje's estimates). If we assume that the ice draft distribution of Fig. 6 is characteristic of the yearly outflow, and that the ice velocity profile is also characteristic in shape although varying in magnitude, we can convert these area figures into transport figures, giving 0.103–0.150 Sv of water equivalent as the mean annual ice flux through Fram Strait. The midpoint of this range, 0.127 Sv, is an upward revision of the accepted value of 0.1 Sv.

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Identification and molecular cloning of the human *Blym* transforming gene activated in Burkitt's lymphomas

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*DNA*s of six Burkitt's lymphoma cell lines contained an activated transforming gene detected by transfection of NIH 3T3 cells. This gene was cloned from a recombinant library of Burkitt's lymphoma DNA and identified as a human homologue of chicken *Blym*-1, the transforming gene detected by transfection of chicken B-cell lymphoma DNA.

ACTIVATED transforming genes have been detected by transfection of NIH 3T3 mouse cells with DNAs from a wide variety of neoplasms, including carcinomas, sarcomas, lymphomas and leukaemias (for a review see ref. 1). In the case of lymphoid neoplasms, DNAs from more than 90% of the human, mouse and chicken lymphomas and lymphoma cell lines which have been investigated transform NIH 3T3 cells with high efficiencies²⁻⁴. In contrast, DNAs of non-neoplastic lymphocytes of these species lack transforming activity, including DNAs of normal chicken B lymphocytes from the same individual birds whose neoplasm DNAs actively induce transformation². Activation of cellular transforming genes therefore seems to occur with a high degree of reproducibility during the development of lymphoid neoplasms, supporting the role of these genes in the disease process.

Investigation of the transforming activity of restriction endonuclease-digested DNAs of human and mouse neoplasms representative of early, intermediate and mature stages of B- and T-lymphocyte development revealed that the same transforming genes were activated in multiple independent neoplasms representative of the same cell type^{3,4}. However, different transforming genes were activated in neoplasms representing different stages of differentiation within each cell lineage^{3,4}. Furthermore, the same patterns of restriction endonuclease susceptibility were observed for the transforming activities of DNAs of mouse and human neoplasms representing the same stage of differentiation, indicating that the different transforming genes detected by transfection of lymphoid neoplasm DNAs were conserved between mouse and man^{3,4}.

The isolation and characterization of a transforming gene detected by transfection of chicken B-cell lymphoma DNA has been described previously⁵. The nucleotide sequence of this gene, designated *ChBlym*-1, indicates that it encodes a small protein of 65 amino acids which shows significant homology to the amino-terminal region of the transferrin family of proteins⁵. Using *ChBlym*-1 as a hybridization probe, it was also shown that this gene belongs to a small gene family which is evolutionarily conserved in both chicken and human DNAs⁵.

Burkitt's lymphomas, like chicken B-cell lymphomas, represent neoplasms of B lymphocytes at an intermediate stage of differentiation which express surface immunoglobulin but do not secrete large amounts of antibody. Epstein-Barr virus (EBV) has been shown to have a role in Burkitt's lymphoma in Africa, where the occurrence of this neoplasm is endemic, although the disease also occurs at lower frequency without any detectable EBV involvement⁶. EBV infection of either normal cord blood lymphocytes or adult peripheral blood lymphocytes results in acquisition of the capacity for continuous B-cell proliferation in culture. However, in contrast to cell lines derived from Burkitt's lymphomas, EBV-immortalized lines of normal lymphocytes are karyotypically normal, polyclonal and unable to form tumours subcutaneously in nude mice⁷. It has

been proposed that EBV infection *per se* will confer only the capacity for continuous proliferation on B lymphocytes and that additional events are required for progression to the full neoplastic phenotype of Burkitt's lymphoma cells⁷.

Here we report the detection of the same activated transforming gene in DNAs from EBV-positive and EBV-negative Burkitt's lymphoma cell lines. This transforming gene has been isolated by molecular cloning and shown to be homologous to *ChBlym*-1.

Transforming activity

DNA of six lymphomas and three lines of EBV-immortalized B lymphocytes were assayed for transforming activity by transfection of NIH 3T3 cells. All of these cell lines exhibited phenotypic characteristics of B lymphocytes at an intermediate stage of differentiation (Ia-positive, B-1-positive⁸ and surface IgM-positive). The Raji and Namalwa African Burkitt's lymphoma cell lines were EBV-positive, whereas the BJAB African Burkitt's lymphoma cell line and all three of the American Burkitt's lymphoma cell lines (CW678, EW36 and MC116) were EBV-negative⁹. All six lymphoma cell lines contained an 8:14 chromosomal translocation⁹. The EBV-immortalized cell lines were established by EBV infection of either normal cord blood lymphocytes (CB-28 and CB-30) or adult peripheral blood lymphocytes (PB-82).

All six lymphoma DNAs induced transformation with high efficiencies (0.2–1.0 transformant per μ g DNA; Table 1). Similar efficiencies of transformation were obtained when NIH cells transformed by these lymphoma DNAs were used as donors of DNA in secondary transfection assays (Table 1). In contrast to the high transforming activity of lymphoma DNAs, all three EBV-immortalized B lymphocyte cell line DNAs lacked transforming activity (Table 1). These results indicate that both EBV-positive and EBV-negative lymphomas contain activated transforming genes detectable by transfection of NIH 3T3 cells. The lack of transforming activity of DNAs of EBV-immortalized lymphocytes indicates that activation of these transforming genes occurs in lymphomas and not in non-neoplastic B cells.

The transforming genes detected by transfection of both EBV-positive and EBV-negative lymphoma DNAs were compared by investigating their sensitivity to cleavage with restriction endonucleases. Transforming activities of DNAs of all six lymphomas were inactivated by digestion with *Bam*HI but not *Eco*RI, *Hind*III or *Xho*I (Table 2). These results indicate that the same transforming gene was activated in each of these six individual lymphomas, whether or not they were associated with EBV. In addition, the restriction endonuclease susceptibility of these human lymphoma DNAs was the same as that previously observed for transforming activity of DNAs of three mouse B-cell lymphomas³, indicating that related transforming

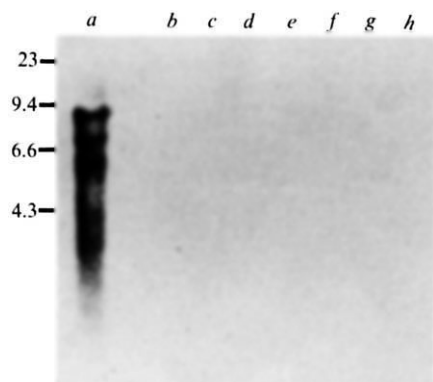


Fig. 1 Analysis of EBV DNA sequences in transformed NIH cells. DNAs (15 µg) were digested with *Bam*HI and analysed by blot-hybridization as described previously³ with ³²P-DNA probe prepared by nick-translation of a pool of plasmid clones containing all of the *Bam*HI fragments of EBV DNA¹⁰. Lane a, Namalwa DNA; b, NIH 3T3 DNA; c–h, DNAs of NIH cells transformed by DNAs of the Burkitt's lymphomas MC116, EW36, CW678, BJAB, Namalwa and Raji, respectively. The filter was autoradiographed for 2 days with an intensifying screen. The sizes of marker fragments of *Hind*III-digested λ DNA are indicated in kilobases.

genes are activated in both human and mouse neoplasms of this cell type.

To determine whether the transforming genes detected by transfection of Burkitt's lymphoma DNAs were linked to EBV DNA sequences, NIH cells transformed by these lymphoma DNAs were analysed by blot-hybridization with a probe prepared by nick-translation of a pool of plasmid clones containing all the *Bam*HI fragments of EBV¹⁰. This probe hybridized to multiple *Bam*HI fragments in DNA of the Namalwa cell line (Fig. 1a), which has been estimated to contain only one or two copies of an incomplete EBV genome¹¹. In contrast, the EBV probe did not hybridize to DNA of NIH 3T3 cells (Fig. 1b) or to DNAs of NIH cells transformed by Burkitt's lymphoma DNAs (Fig. 1c–h). These results indicate that the transforming genes of EBV-positive Burkitt's lymphomas (Raji and Namalwa) are not linked to EBV genomes, although the possibility of linkage to a small fragment of viral DNA cannot be excluded due to the genomic complexity (~100 kilobases (kb)) of EBV.

Molecular cloning

Burkitt's lymphomas are neoplasms representing the same stage of B-cell differentiation as the chicken B-cell lymphomas from which a transforming gene (*ChBlym-1*) has been isolated and characterized⁵. Because the *ChBlym-1* transforming gene is homologous to a small family of evolutionarily conserved genes present in human as well as chicken DNA⁵, it was of interest to investigate the possibility that a human homologue of *ChBlym-1* might correspond to the transforming gene detected by transfection of Burkitt's lymphoma DNA. Molecular clones of Burkitt's lymphoma DNA homologous to *ChBlym-1* were therefore isolated and tested for transforming activity.

DNA prepared from the CW678 lymphoma cell line was partially digested with the restriction endonuclease *Mbo*I and 15–25-kb fragments were cloned in the *Bam*HI site of the λ vector L47.1 (ref. 12), packaged *in vitro*¹³ and amplified by plate lysis on *Escherichia coli* strain 1160. Plaque size on this strain was small, so recombinant phage were then amplified on *E. coli* strain C600, yielding significantly larger plaques. 140,000 phage were amplified in this manner and screened by *in situ* hybridization¹⁴ using a ³²P-labelled M13 probe representing the 3' half of the *ChBlym-1* gene (M13R9, ref. 5). The hybridization and washing conditions used were of moderate stringency, as described in Fig. 2 legend. Fifteen positively hybridizing clones were detected, plaque-purified and amplified in liquid culture for further study.

Table 1 Transforming activity of Burkitt's lymphoma DNAs

Donor DNA	Total foci/total recipient cultures	Foci per µg DNA
EBV-positive lymphomas		
Raji	73/14	0.26
Namalwa	47/14	0.17
EBV-negative lymphomas		
BJAB	81/14	0.29
CW678	280/14	1.0
EW36	98/14	0.35
MC116	64/14	0.23
EBV-immortalized lymphocytes		
CB-28	0/10	<0.005
CB-30	0/10	<0.005
PB-82	0/10	<0.005
Transformed NIH cells		
NIH(Raji) (3)	64/18	0.18
NIH(BJAB) (2)	45/12	0.19
NIH(Namalwa) (3)	61/18	0.17
NIH(CW678) (3)	283/18	0.78
NIH(EW36) (2)	53/12	0.22
NIH(MC116) (3)	66/18	0.18

NIH 3T3 cells were exposed to 20 µg of donor DNA per recipient culture and foci of transformed cells were counted 11–13 days after transfection³. Lymphoma cell lines and immortalized lymphocyte cell lines CB-28 and CB-30 were obtained from I. Magrath⁹. Two or three independent foci (indicated in parentheses) of NIH cells transformed by each of the Burkitt's lymphoma DNAs were grown to mass cultures and used as donors of DNA in secondary transfection assays. Independent foci from the same original lymphoma DNA gave similar transformation efficiencies. The background of spontaneous transformation in control cultures exposed to salmon sperm, NIH 3T3 or normal human embryo fibroblast DNA was less than 0.005 transformant per µg.

Table 2 Restriction endonuclease digestion of Burkitt's lymphoma DNAs

Donor DNA	Foci per µg DNA				
	Undigested	<i>Eco</i> RI	<i>Hind</i> III	<i>Bam</i> HI	<i>Xho</i> I
Raji	0.22	0.23	0.25	0.01	0.28
BJAB	0.28	0.26	0.29	<0.01	0.26
Namalwa	0.25	0.18	0.18	0.01	0.18
CW678	1.30	1.2	1.30	0.01	1.30
EW36	0.33	0.35	0.33	<0.01	0.35
MC116	0.23	0.27	0.24	0.01	0.24

DNAs (80 µg) of Burkitt's lymphomas were digested to completion with the indicated restriction endonucleases. Transforming activities of undigested and digested DNAs were assayed by transfection of NIH 3T3 cells as described in Table 1 legend.

Each clone was tested for transforming activity by transfection of NIH 3T3 cells with phage particles⁵. One of the 15 clones induced transformation with high efficiency ($2-3 \times 10^4$ transformants per pmol of DNA) whereas the other clones lacked detectable transforming activity. This actively transforming clone was designated λHuBlym-1 and used for further studies. The transforming activity of λHuBlym-1 is similar to that observed for molecular clones of other activated transforming genes.

Characterization of λHuBlym-1

The cellular DNA insert of λHuBlym-1 was ~14 kb. To localize the sequences homologous to *ChBlym-1*, restriction endonuclease digests of λHuBlym-1 DNA were analysed by blot-hybridization using ³²P-labelled probe containing the 3' half of *ChBlym-1* (R9, ref. 5). As illustrated in Fig. 2, restriction endonuclease digests resulting in single fragments homologous to *ChBlym-1* included *Eco*RI (0.95 kb), *Hind*III (2.2 kb) and *Hind*III + *Bam*HI (1.6 kb). The same fragments of λHuBlym-1 were detected by blot-hybridization with a probe containing the 5' half of *ChBlym-1* (R16, ref. 5) (data not shown).

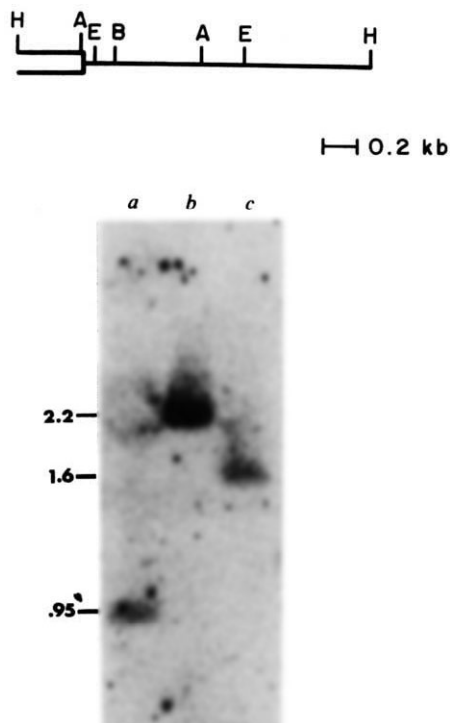


Fig. 2 Restriction map of the region of λ HuBlym-1 homologous to *ChBlym-1*. Top: Cleavage sites for *Hind*III (H), *Ava*I (A), *Eco*RI (E) and *Bam*HI (B) are localized in the 2.2-kb *Hind*III fragment of λ HuBlym-1 which hybridizes to the *ChBlym-1* probe. This fragment contains 0.5 kb of vector DNA ($\Rightarrow$) linked to 1.7 kb of cellular DNA ($-$). Bottom: λ HuBlym-1 DNA was digested with *Eco*RI (lane a), *Hind*III (b) or *Hind*III + *Bam*HI (c) and analysed by blot hybridization with probe prepared by nick-translation of a plasmid (pR9) containing the 3' half of *ChBlym-1* (ref. 5). Hybridization and washing conditions were as described elsewhere¹⁴ except that the filter was washed twice with $4 \times$ SET, 0.5% SDS ($1 \times$ SET = 0.15 M NaCl, 30 mM Tris-HCl, 1 mM EDTA pH 8.0) for 2 h at 68 °C and rinsed 10 times in 3 mM Tris base, 0.1 M NaCl at room temperature. Autoradiographic exposure was for 4 days with an intensifying screen. The sizes of hybridizing fragments were determined relative to marker fragments of *Hind*III-digested λ DNA and are indicated in kilobases.

To further characterize the region of λ HuBlym-1 which was homologous to *ChBlym-1*, the 2.2-kb *Hind*III fragment of λ HuBlym-1 was purified by agarose gel electrophoresis. Cleavage sites for *Ava*I, *Bam*HI and *Eco*RI were mapped within this fragment by using a nick-translated 32 P-labelled preparation of the 2.2-kb *Hind*III fragment as probe for blot-hybridization analysis of single and double digests of λ HuBlym-1 DNA. The restriction map of this region of λ HuBlym-1 is presented in Fig. 2. The 2.2-kb *Hind*III fragment is a phage-cell junction fragment containing ~ 0.5 kb of phage DNA. The 0.95-kb *Eco*RI fragment is an internal fragment homologous to *ChBlym-1* and contains the single *Bam*HI site ~ 140 nucleotides from its left-hand terminus.

To determine whether the transforming sequences and the *ChBlym-1* homologous sequences of λ HuBlym-1 were the same, the transforming activity of the gel-purified 2.2-kb λ HuBlym-1 *Hind*III fragment was assayed by transfection. This fragment induced transformation with the same molar efficiency as intact λ HuBlym-1 DNA (Table 3), localizing the transforming gene of λ HuBlym-1 to the same *Hind*III fragment which contained the *ChBlym-1*-homologous sequences.

The purified 2.2-kb *Hind*III fragment of λ HuBlym-1 was also used to assess the sensitivity of the cloned transforming gene to digestion with a series of restriction endonucleases. Cleavage of this fragment with the enzymes *Hind*III (included as a control), *Eco*RI and *Xho*I did not affect transforming activity, whereas the isolated transforming gene was inactivated

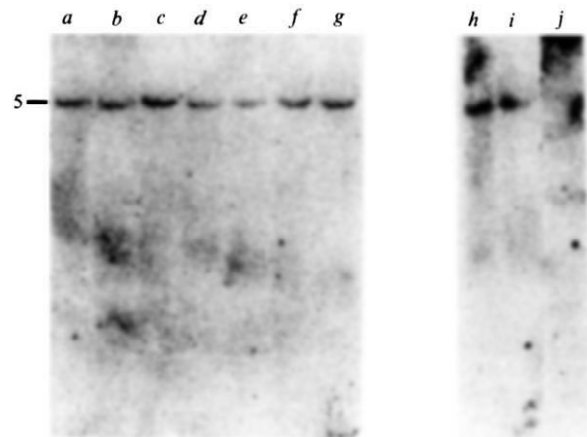


Fig. 3 Analysis of *HuBlym-1* sequences in cellular DNAs. Cellular DNAs (15 μ g) were digested with *Hind*III and analysed by blot-hybridization with probe prepared by nick-translation of the agarose gel-purified 0.95-kb *Eco*RI fragment of λ HuBlym-1 DNA. Hybridization and washing conditions were as described previously¹⁴ except that the filter was washed in $0.25 \times$ SET, 0.5% SDS at 68 °C. Lane a, normal human embryo fibroblast DNA; lanes b–g, DNAs of Burkitt's lymphoma cell lines CW678, MC116, EW36, Raji, BJAB and Namalwa, respectively; h, i, DNAs of NIH cells transformed by CW678 and EW36 lymphoma DNAs; j, DNA of NIH 3T3 cells. The filter containing lanes a–g was autoradiographed overnight without an intensifying screen, whereas that containing lanes h–j was autoradiographed for 2 days with an intensifying screen. The size of the hybridizing fragment is indicated in kilobases.

by *Bam*HI digestion (Table 3). This pattern of restriction endonuclease susceptibility exactly matches the pattern observed for transforming activity of total Burkitt's lymphoma DNAs (Table 2). This indicates that the λ HuBlym-1 transforming gene, isolated from a recombinant library of Burkitt's lymphoma DNA by hybridization to the chicken B-cell lymphoma transforming gene, is the same gene as that detected by transfection of total Burkitt's lymphoma DNA. Furthermore, the sensitivity of the transforming activity of the λ HuBlym-1 *Hind*III fragment to digestion with *Bam*HI, but not *Eco*RI, localizes the transforming gene of λ HuBlym-1 to the 0.95-kb *Eco*RI fragment which hybridizes to the *ChBlym-1* probe (Fig. 2), thus indicating that the *ChBlym-1*-homologous sequence contains the observed transforming activity. As digestion with *Eco*RI does not inactivate transforming activity, the size of the *HuBlym-1* gene is less than 0.95 kb.

The 2.2-kb *Hind*III fragment containing the transforming gene was also used to determine whether the *HuBlym-1* gene was homologous to the transforming genes of retroviruses. The fragment was 32 P-labelled by nick-translation and hybridized to molecular clones of the viral transforming genes *myc*, *Ha-ras*, *Ki-ras*, *fms*, *erb*, *myb*, *abl*, *src*, *sis*, *fes*, *mos* and *rel*. No hybridization was observed to any of these clones using the conditions of moderate stringency described in Fig. 2 legend. This is consistent with the previous observation that *ChBlym-1* was similarly not homologous to any of these retroviral transforming genes⁵.

Cellular *HuBlym-1* sequences

The 0.95-kb *Eco*RI fragment containing the *HuBlym-1* transforming gene was used as a probe for blot-hybridization analysis of cellular DNAs. Using stringent conditions of hybridization, this probe hybridized to a major *Hind*III fragment of ~ 5 kb in DNAs of both normal human embryo fibroblasts and Burkitt's lymphomas (Fig. 3a–g). The transforming potential of *HuBlym-1*

Table 3 Localization and restriction endonuclease digestion of the λ HuBlym-1 transforming gene

Donor DNA	Restriction endonuclease	Transforming activity (foci per pmol DNA)
λ HuBlym-1	—	2.8×10^4
2.2-kb <i>Hind</i> III fragment	—	3.0×10^4
2.2-kb <i>Hind</i> III fragment	<i>Hind</i> III	3.6×10^4
2.2-kb <i>Hind</i> III fragment	<i>Eco</i> RI	3.6×10^4
2.2-kb <i>Hind</i> III fragment	<i>Xho</i> I	4.7×10^4
2.2-kb <i>Hind</i> III fragment	<i>Bam</i> HI	$<10^3$

Transforming activities of intact λ HuBlym-1 DNA and of the 2.2-kb λ HuBlym-1 *Hind*III fragment homologous to *ChBlym-1* were assayed by transfection of NIH 3T3 cells. The 2.2-kb *Hind*III fragment was purified by agarose gel electrophoresis of *Hind*III-digested λ HuBlym-1 DNA and was assayed either without further restriction endonuclease digestion or after subsequent digestion with *Hind*III (as a control), *Eco*RI, *Xho*I or *Bam*HI. Recipient cultures of NIH 3T3 cells were exposed to 1 ng of purified fragment DNA, or to 20 ng of intact phage DNA, in the presence of 20 μ g of NIH carrier DNA. Foci were counted 12–14 days after transfection. Results are presented as foci per pmol DNA to facilitate comparison of the transforming activities of purified fragment and intact phage DNAs.

1 in lymphomas therefore did not appear to be activated as a consequence of either gene amplification or DNA rearrangements. A similar *Hind*III fragment was detected in DNAs of NIH cells transformed by Burkitt's lymphoma DNAs (Fig. 3*h*, *i*) but not in DNA of NIH 3T3 cells (Fig. 3*j*). These results further demonstrate that *HuBlym-1* is the transforming gene detected by transfection of total cellular DNAs of Burkitt's lymphomas.

Conclusions

We have detected the same activated transforming gene in DNAs of six Burkitt's lymphoma cell lines. This transforming gene, designated *HuBlym-1*, was isolated as a molecular clone by virtue of its homology to a transforming gene, *ChBlym-1*, previously isolated from NIH cells transformed by chicken B-cell lymphoma DNA. Because *HuBlym-1* was isolated as an active transforming gene directly from a recombinant library of Burkitt's lymphoma DNA, the possibility that it was activated as a consequence of transfection, rather than in the original neoplasm, can be excluded. In addition, analysis of *HuBlym-1* by restriction endonuclease mapping and blot-hybridization indicates that the cloned transforming gene is the same as that detected by transfection with total cellular DNA of Burkitt's lymphomas. These results establish that homologous transforming genes are activated in B-cell lymphomas of both chicken and human origin. The reproducibility and high degree of species conservation observed for activation of *Blym-1* in these lymphomas provides strong support for its role in neoplastic disease.

Human *Blym-1*, like chicken *Blym-1* (ref. 5), was not homologous to molecular clones of any retroviral transforming genes tested, including *Ha-ras* and *Ki-ras*, which have been identified as activated transforming genes in a variety of human neoplasms^{15–19}. In addition, its restriction map and lack of homology to *Ha-ras* and *Ki-ras* distinguish *Blym-1* from the

recently described *N-ras* transforming gene^{20,21}. *Blym-1* therefore does not appear to be a member of the *ras* family.

Mapping of the *HuBlym-1* transforming gene indicates that, like *ChBlym-1*, it is small (<1 kb) and the nucleic acid sequence homology between the chicken and human genes implies that they encode closely related gene products. The possibility that the transforming activity of the *ChBlym-1* protein might be functionally related to growth factor activities of transferrin has been discussed previously⁵.

B-cell lymphomas in chickens are induced by infection with lymphoid leukemia viruses, members of a class of retroviruses which do not contain viral transforming genes and induce neoplasms having long latent periods. Induction of lymphomas by these viruses involves integration of viral transcriptional regulatory sequences in the vicinity of the cellular gene (*c-myc*) homologous to the transforming gene of avian myelocytomatosis virus, resulting in increased *c-myc* expression²². However, the transforming gene detected by transfection of chicken B-cell lymphoma DNAs, *ChBlym-1*, is not linked to viral DNA sequences² and is not homologous to *c-myc* (refs 5, 23). Thus, at least two distinct transforming genes are implicated in the development of chicken B-cell lymphomas. As the pathogenesis of these lymphomas suggests a multi-step process including both pre-neoplastic and neoplastic stages²⁴, an attractive hypothesis is that *c-myc* and *Blym-1* may be involved in different stages of progression to the neoplastic phenotype.

Burkitt's lymphoma is similarly thought to be the consequence of a multi-step process. Infection of normal lymphocytes with EBV leads to the outgrowth of immortalized B lymphocyte cell lines which are capable of continuous growth in culture but are not tumorigenic in nude mice⁷. As these immortalized cell lines may represent an early pre-neoplastic stage of lymphomagenesis, it is of interest that their DNAs do not induce transformation on transfection, indicating that the transforming activity of *HuBlym-1* is not activated at this stage of the disease process. In addition, Burkitt's lymphomas, including those used in the present study, are associated with chromosomal translocations^{25,26} which have recently been found to occur in the region of *c-myc* (refs 27–30). These findings indicate that translocations of *c-myc* are associated with the development of Burkitt's lymphomas and, taken together with the present report, imply that the same two transforming genes, *c-myc* and *Blym-1*, are involved in B-cell lymphomas of both chicken and man.

Activation of multiple transforming genes has also been observed in neoplasms other than B-cell lymphomas, including virus-induced mouse mammary carcinomas^{31,32}, Abelson virus-induced mouse pre-B-cell lymphomas⁴ and mouse plasmacytomas^{1,3,33–36}. Although the role of these genes in carcinogenesis remains to be established, it is an attractive hypothesis that the reproducible activation of multiple transforming genes in neoplasms may be correlated with the multi-step progressive nature of neoplastic disease.

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LETTERS TO NATURE

Rotation of Venus's polar dipole

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The venusian polar dipoles are long-lived, elongated, warm features seen in images of thermal emission from the polar cloud tops of the planet. They are almost 4,000 km across, are centred close to the pole, and appear to rotate with a period of ~3 days retrograde. The northern hemisphere dipole was first identified as such by the Orbiter Infrared Radiometer (OIR) on the Pioneer Venus Orbiter (PVO)^{1,2}, and in this study we use OIR images at 11.5 μm to investigate its detailed rotation. Its rotation rate is observed to change steadily over the 72-day data set, and there is some evidence for oscillatory variations superimposed on this trend.

On 4 December 1978 the PVO spacecraft was placed in a high-inclination 24-h orbit around Venus. Throughout its 72 days of operation the OIR obtained good coverage of the northern hemisphere of the planet from this platform, particularly the north polar region not easily accessible to Earth-based observations. The OIR instrument³ contains six thermal channels. Four, near the 15- μm carbon dioxide band, are sensitive to emission from atmospheric layers roughly 10 km thick centred at 70, 80, 90 and 100 km, and the other two are sensitive to cloud-top emission at 11.5 and 45 μm .

Figure 1 shows OIR images of the northern hemisphere of Venus for nine contiguous PVO orbits. These display the difference in brightness temperature between the 11.5- and 13.7- μm (80 km) channels. Because the former channel can see the dipole whereas the latter cannot, differencing enhances contrasts and eliminates some of the effects of limb-darkening. The dipole is seen as a warm (bright), elongated, wavenumber 2 structure extending to 70° N with distinct hot spots centred near 75° N. It is surrounded by the cold (dark) polar collar, and contrasts in 11.5- μm brightness temperature between the collar and dipole can exceed 35 K at 75° N. Part of the dipole was first observed by Taylor *et al.*⁴ and its morphology has been discussed elsewhere^{1,2}. The present work is an extension of preliminary results reported by Diner⁵, and its chief aim is to describe the rotation of the dipole over the available 72-day data set.

On a day-to-day basis the structure of the dipole is variable, although its gross appearance and rotation rate are fairly stable. In solar-fixed coordinates it appears to rotate in a retrograde (clockwise) sense with a period of ~3 days. The images of Fig.

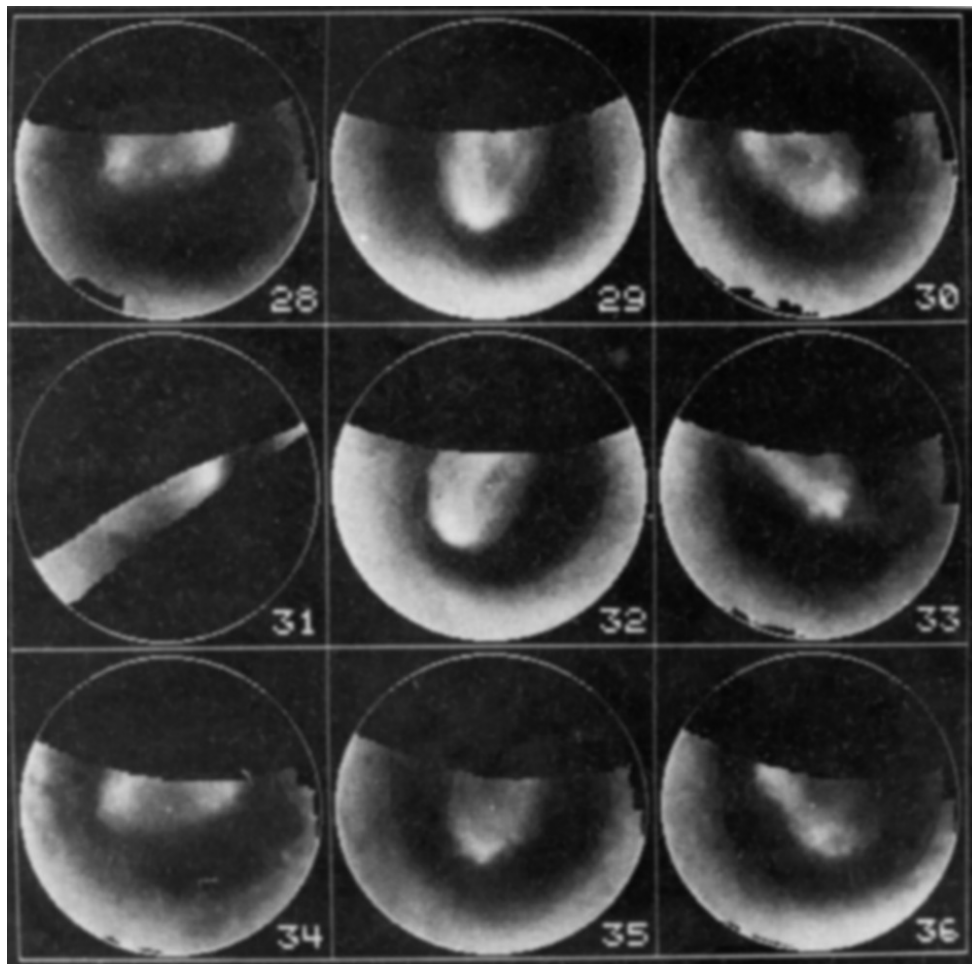
1 are organized in 3-day rows to emphasize this period, and rotation rate fluctuations and changes in morphology may be seen by comparing vertical columns. In the absence of asymmetry in the dipole, and bearing in mind the 1-day sampling rate, the images of Fig. 1 are consistent with retrograde rotation rates of $R(N) = -\Omega - (N-1) \times 180 \text{ deg day}^{-1}$ and prograde rates of $P(N) = 180 - \Omega + (N-1) \times 180 \text{ deg day}^{-1}$, where N is an integer with values 1, 2, 3, ... and Ω is the apparent retrograde rotation between adjacent images (~120 deg day⁻¹). In practice, asymmetries in hotspot intensity can be followed for several orbits allowing the even N terms in $R(N)$ and the odd N terms in $P(N)$ to be eliminated. The absence of smearing in the finite time required to form a dipole image also places an upper limit of $N = 5$ on both series. Although we cannot rule out the remaining possibilities, the retrograde rate $R(1)$ is chosen because it corresponds to the slowest phase speed relative to the 4–5 day bulk retrograde rotation of the atmosphere observed at comparable levels and lower latitudes^{6,7}.

Given the choice of $R(1)$, the orientation of the dipole relative to a coordinate system rotating uniformly at this rate can be followed through images covering the entire data set. However, because it is not always centred on the pole and as its morphology is variable, the assignment of a longitude to one arm of the dipole may suffer from observational bias. Therefore we performed measurements separately, using independently calibrated data and different imaging techniques, coordinate systems, dipole definitions, and methods of measurement. Dipoles were identified in 66 out of 72 orbits, 49 of which were common to both sets of analyses. Missing dipoles were due solely to incomplete data. Measurement errors in orientation, obtained by combining the data sets, were generally estimated to be 7°, and in only 2 out of the 49 shared orbits did differences between the data sets exceed these errors.

Figure 2 shows an image constructed by averaging all the 11.5- μm images for individual orbits in a dipole-fixed coordinate system. The pole is identical to the solar-fixed pole, zero longitude is the measured dipole orientation, and brightness temperatures range from 242 K (bright) to 217 K (dark). The mean structure of the dipole can be seen clearly. Symmetrical hotspots are joined by a warm band across the pole and the circular collar, which extends to 60° N, is most intense at right angles to the dipole.

The mean value of $R(1)$, derived from a least-squares, straight line fit to the combined sets of measured dipole orientation longitudes, gives a period of -2.945 ± 0.003 days in solar-fixed coordinates ($\Omega = 122.23 \pm 0.12 \text{ deg day}^{-1}$). This period becomes -2.985 and -3.022 days respectively in celestial and body-fixed coordinates. The minus sign indicates retrograde rotation and the error represents the propagation of measurement error through the fitting algorithm. Figure 3a shows longitude residuals for this fit. It is evident that these exceed measurement errors although day-to-day scatter does not. Features in the residual curve, such as those following day 10 and preceding day 50, are seen in both independent data sets

Fig. 1 North polar images for orbits 28–36 obtained by subtracting $13.7\text{ }\mu\text{m}$ (80 km) brightness temperatures from $11.5\text{-}\mu\text{m}$ cloud top values. The projection is polar stereographic, solar-fixed, and extends to a minimum lat. of 55°N . Local noon is at the bottom of each circle and the morning terminator is to the right. The cool collar region (dark) and the rotating, warm dipole (bright) are evident. Note the transient nature of features within the dipole (compare orbits 29, 32, and 35) and occasional drifts away from the pole (such as orbit 32). The apparent discontinuity bordering the collar region is an artefact of the video display, and sharp edges mark the limits of coverage.



and are considered real. The simplest explanation of the residual curve is that $R(1)$ changes abruptly near day 28. However, its parabolic appearance suggests that the data might be better fitted by a quadratic curve.

Figure 3b shows the longitude residual curve corresponding to the quadratic part of a least-squares, quadratic plus cosine fit. Its periodic character, significantly above measurement error, originally suggested the addition of a cosine term to the fit. The optimum 40 ± 1 -day period of this term was found by a systematic frequency search, which gave a standard deviation of 10.1° in the residuals for the composite fit. This approaches the 7° expected from measurement error alone and is not improved appreciably by higher-order fits.

The second time differential of the composite fit to $R(1)$ of Fig. 3b gives a steady deceleration term of $0.21 \pm 0.05 \text{ deg day}^{-2}$. Superimposed on this overall drift is an oscillating term with a period of ~ 40 days and a peak amplitude of $0.39 \pm 0.06 \text{ deg day}^{-2}$, although a much longer data set is needed to verify its regularity. The steady term produces a 10% deceleration over the 72-day data set, corresponding to periods of -2.72 and -3.13 days at orbits 1 and 72 respectively, and suggesting that the dipole would cease rotation 627 ± 151 days after orbit insertion. However, a literal interpretation of this extrapolation from the comparatively short data set is probably unwarranted. The above fit applies to $R(1)$, but the behaviour of $R(N)$ and $P(N)$ in terms of $R(1)$ can be found easily from the value of θ and the series given earlier.

A quantitative description of the longitudinal structure of the dipole can be obtained by expressing the variation of brightness temperature around latitude circles in terms of Fourier series. Figure 4 shows the latitude dependence of the first four Fourier component amplitudes derived from an $11.5\text{-}\mu\text{m}$ dipole-fixed image similar to Fig. 2. Second component, or wavenumber 2, amplitudes are dominant wherever longi-

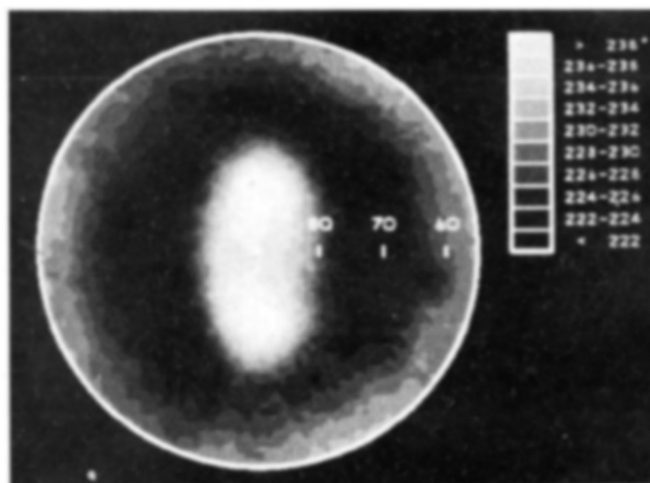


Fig. 2 The mean dipole-fixed $11.5\text{-}\mu\text{m}$ brightness temperature of the northern hemisphere of Venus from 55 to 90°N over a period of 72 days. Latitude is specified in solar-fixed coordinates, but longitude is defined relative to the dipole, and a circular polar projection is used. Each grey-scale contour represents a temperature contrast of 2 K. This image is constructed by averaging images similar to those of Fig. 1 in dipole-fixed longitude coordinates.

tudinal variations are significant, and are symmetrically distributed about an 8-K peak at 77°N with a width at half-maximum of 10.5° lat. Wavenumbers 1 and 4 are also present with maximum amplitudes near 2 K at 75°N . Wavenumber 1 is responsible for small asymmetries in the dipole and wavenumber 4 for its elongation. Wavenumber 3 amplitudes, however, do not exceed the measurement noise level.

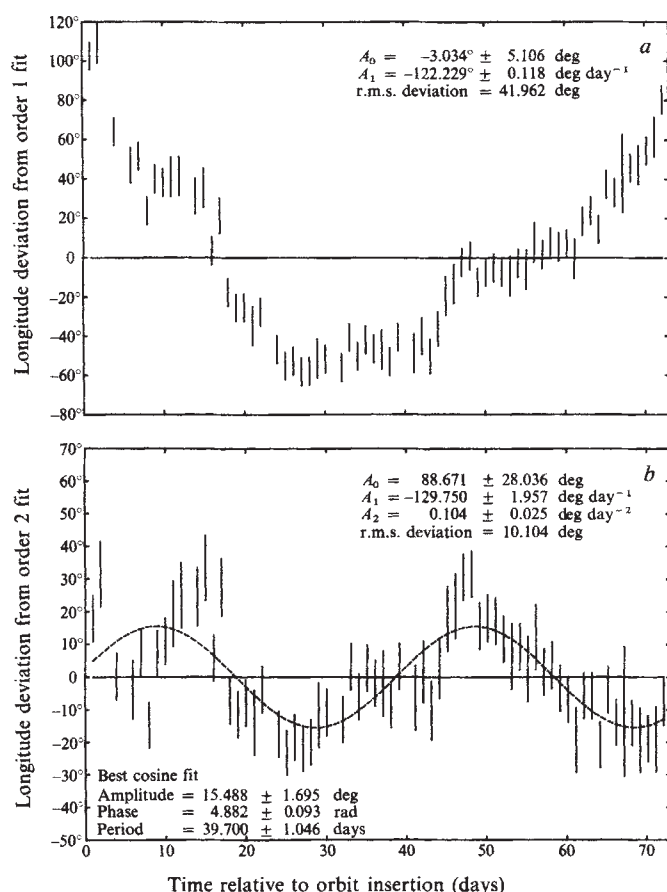


Fig. 3 *a*, The deviation of the combined dipole orientation data from a least-squares, straight line fit of the form $\theta = A_0 + A_1 t$, where θ (deg) is the dipole longitude and t (days) is the time. Solar-fixed longitudes are used, the fit is represented by the solid line, and the vertical bars represent errors in the longitude measurements. The fit corresponds to a mean rotation rate -2.945 ± 0.003 days. *b*, The deviation of the same data from the quadratic component of a composite least-squares quadratic plus cosine fit of the form $\theta = A_0 + A_1 t + A_2 t^2 + B \cos(2\pi t/T + \phi)$, where B (deg) is the amplitude, T (days) is the period, and ϕ (rad) is the phase. Solid line, the polynomial; dashed line, the cosine fit; vertical bars, the errors in longitude measurement.

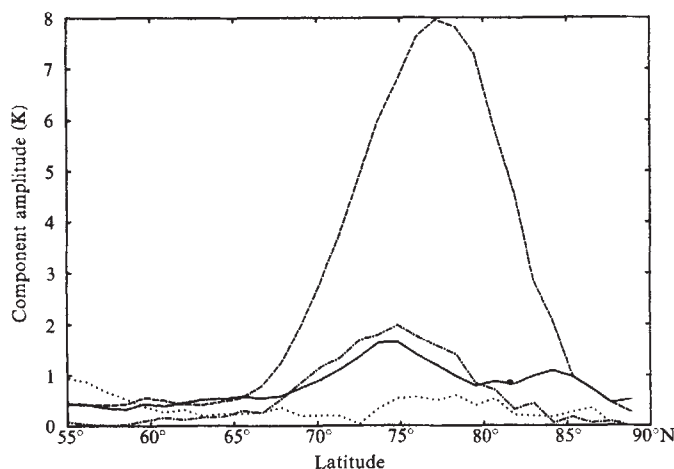


Fig. 4 The latitude dependence of the wavenumber 1 (solid line), 2 (dashed line), 3 (dotted line), and 4 (dot-dashed line) amplitude components derived from the Fourier analysis of the variation of 11.5- μm brightness temperature around latitude circles in an image similar to Fig. 2. This image has been corrected for limb-darkening by subtracting the mean image in celestial coordinates from individual images before averaging in dipole-fixed coordinates.

In spite of its intensity in OIR images at 11.5 and 45 μm , the atmospheric structure responsible for the dipole is uncertain. Because it does not appear in UV, visible or near-IR images, or in polarization measurements, Schubert *et al.*⁸ argue that it is a temperature rather than a cloud feature. However, Kawabata *et al.*⁹ show that the poles are shrouded by a submicrometre haze which could hide the dipole from reflected solar radiation, especially when the oblique angle of illumination is considered. A Mariner 10 UV image of the south polar cap¹⁰ shows a vortex with some elliptical (wavenumber 2) structure, but its relevance to the dipole is unclear as it is a composite based on an assumed 4-day rotation period and covering several days, during which the more rapidly rotating dipole would be smeared out. OIR and radio occultation measurements from the PVO^{11,12} have demonstrated that the collar is produced by a deep cloud top temperature inversion. Detailed comparisons of the data sets¹³ indicate that this inversion fills and the cloud top sinks as a dipole hotspot is approached, suggesting that the dipole is a combination of temperature and cloud phenomena. Finally, its attenuation by a factor of 3 in 13.1- μm (70-km) images and its absence from 13.7- μm (80-km) images means that either any dipole temperature component decays rapidly above the clouds or the vertical wavelength is small compared with the vertical resolution of these channels.

The periodicity of the dipole and its long-term variation must be linked to the circulation of the cloud top atmosphere. At this level UV cloud tracking has established that hemispherical vortex regimes apply equatorward of 60°, consistent with a Hadley circulation in which rapid retrograde zonal flows are superimposed on weaker poleward motion^{6,7}. In Mariner 10 images, rotation periods vary from -4.5 days at low latitudes to a 'dipole-like' -3.0 days in a mid-latitude jet near 50°N. However, this jet is much weaker in PVO images⁷, although the PV north probe measured winds at 60°N consistent with a 3-day period above 50 km (ref. 14). Studies of fluctuations in OIR brightness temperatures¹⁵ reveal 5.3 ± 0.4 -day components from the cloud tops to 90 km, with amplitudes that increase with latitude to maxima at 60–70°N. Similar studies of PVO UV images¹⁶ reveal 3.9- and 5.2-day components, the latter being dominant at mid-latitudes. One interpretation of the UV results is a 30^{+30}_{-10} day modulation in some property of features moving with the 4.5-day wind. Perhaps this is related to the 40-day modulation of the dipole rotation rate presented here.

The steady deceleration of the dipole requires circulation changes with time constants in excess of 100 days. This is surprising considering the lack of seasonal forcing on Venus, but is supported by the cloud top circulation changes between the Mariner 10 and Pioneer Venus missions and by other long-term trends. In particular the OIR channels show that it is accompanied by a slow drift in the thermal structure of the polar atmosphere. Within 5° of the pole, cloud top temperatures fall by 10 K, those at 80 km fall by 2–3 K, and those at 90 km rise by 10 K over the 72-day observation period. In a comparable overlapping period, the disk-integrated UV brightness of Venus appears to fall and polar brightness varies on a time scale of a few months¹⁶. Earth-based UV and thermal images show similar changes^{17,18}. Both the collar and dipole have been seen from Earth^{19,20}, although oblique viewing geometry prevents their accurate description and the simultaneous observation of the dipoles. However, both collars can be seen and these display considerable variability¹⁸.

Although several ideas are being pursued, the circulation patterns and mechanisms responsible for the dipole are presently not uniquely constrained by the observations. R. Hide has suggested (personal communication) that the dipole may be caused by a double vortex in the descending arm of the hemispherical circulation, which might be more efficient than a single vortex in the vertical transport of angular momentum. Studies of the normal modes of the polar atmosphere, to determine whether the dipole is a resonant state, are underway^{21,22}. Elson²³, has shown that the dipole morphology and phase speed

are consistent with barotropic instabilities associated with a narrow high latitude jet produced by the polar collar temperature inversion, wavenumber 2 instabilities having the greatest rate of growth. Recently, Leovy²⁴ has suggested that the zonally symmetric circulation regime extends only to 60° lat., implying that the dipole is not involved in the primary Hadley circulation. In all these cases work is restricted by a limited knowledge of global temperature and wind fields below the cloud tops. An improved description of the dipoles, particularly in the long-term, as well as a better theoretical understanding, awaits a future Venus polar orbiter mission.

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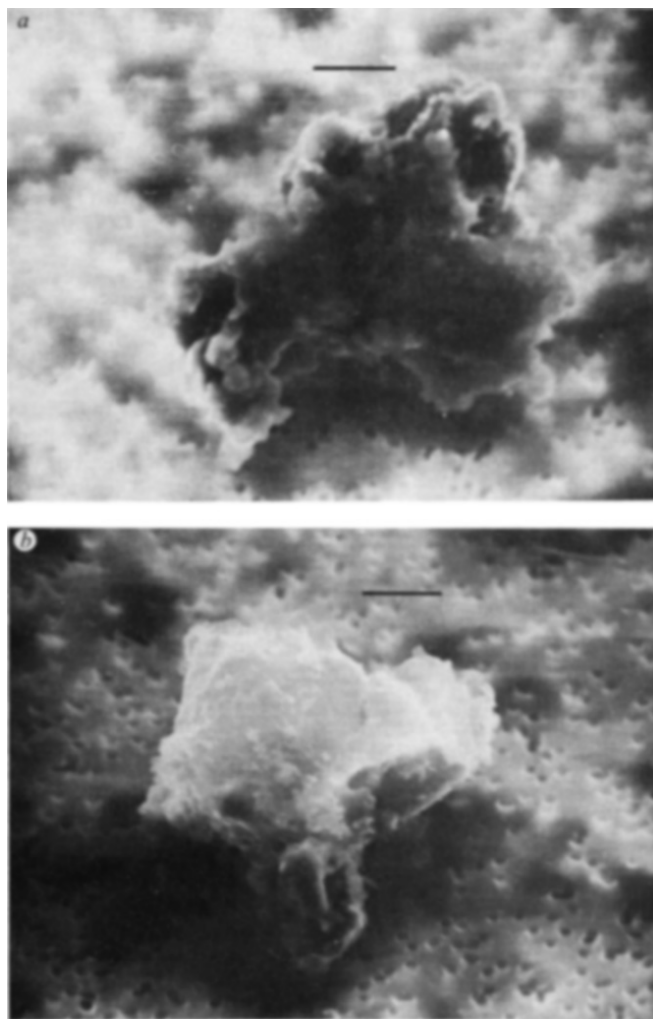


Fig. 1 SEM micrographs of stratospheric dust particles r21-M3-5B (a) and r21-M4-7 (b). Scale bars, 5 μ m.

Laboratory measurements of D/H ratios in interplanetary dust

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Measurements of noble gas elemental and isotopic abundance patterns have provided evidence that a subset of the particles collected in the upper atmosphere by NASA aircraft are micrometeorites. This 'chondritic' subset is characterized by major and trace element contents within a factor of 2 of cosmic abundances¹. We have found that the deuterium in two such particles is enriched relative to hydrogen by 500–1,100% when compared with the terrestrial SMOW standard. This result confirms the extraterrestrial origin of the 'chondritic' dust particles and demonstrates that the material preserves an interesting, and potentially unique, isotopic record which may reflect a memory of processes occurring during (or before) the formation of the Solar System. In addition, the deuterium enrichment seen in the interplanetary dust particles is analogous to that observed in bulk samples of one carbonaceous meteorite and two unequilibrated ordinary chondrites as well as in chemical separates of a number of other meteorites. In this sense interplanetary dust is also 'primitive' material.

The measurements were performed with a CAMECA IMS-3F ion microprobe recently installed at Washington University. Negative secondary ions sputtered from the samples by Cs⁺ bombardment were analysed. The analysis of negative

deuterium ions has an important advantage over positive ion analysis. High mass-resolution measurements showed that the background of H₂⁺ is <0.5% of the D⁺ signal for all samples; in contrast, positive ion analysis gives an H₂⁺ signal of up to 100 times the D⁺ value. Because of the low interference, our measurements of D⁺/H⁺ were made at low mass resolution ($m/\Delta m = 500$). The yield of D⁺ was also enhanced over D⁺ ions leading to typical count rates of 15–50 counts s⁻¹. Isotopic ratio determinations were made in an automatic peak jumping mode. The exact peak positions were established at the beginning of each run by determining the magnetic field values corresponding to the half-maxima of both mass peaks. The position of the mass 1 peak was tracked during the run and the magnetic field values of both mass 1 and 2 peaks adjusted to keep the ion beams centred in the middle of the exit slit of the mass spectrometer. Counts were corrected for dead time (37.3 ns as determined by independent measurements on a Ti standard).

Sample charging effects can be a serious problem in negative secondary ion analysis of a positive ion bombarded insulating sample. To minimize these effects, individual particles, typically 10–20 μ m in size, were pressed into a gold foil. In addition, the energy distribution of the secondary ions, collected through an energy window of 120 V, was automatically monitored during each run and any sample charging was compensated for by offsetting the accelerating voltage.

To provide calibration standards, two types of terrestrial basalts and one variety of amphibole were broken into small

fragments and mounted in close proximity to the extraterrestrial samples. In addition to the stratospheric dust particles, fragments of two meteorites, Renazzo and Semarkona, were analysed. Measurements on the stratospheric dust particles and the meteorite grains were alternated with those on the terrestrial standards, keeping all probe conditions as constant as possible.

The experimental results are summarized in Table 1. The D/H ratios for terrestrial materials have apparent δD values

$$\delta D = \left[\frac{(D/H)_{\text{sample}}}{(D/H)_{\text{standard}}} - 1 \right] \times 1,000$$

ranging from -130 to $+75$. Deviations from the zero terrestrial SMOW value of $D/H = 0.00015576$ (ref. 2) are partly real (typical δD values of terrestrial samples lie in the range -160 to $+20$)² but are also partly due to mass fractionation effects in the probe itself. Some variability in the mass fractionation is evidenced by the changes in the measured values for individual terrestrial grains and differences in the values found for different grains for the same whole rock. However, the D/H ratios for the meteoritic samples and the stratospheric dust particles are very different from those in the terrestrial samples and lie far outside the range of any instrumental mass-fractionation effects. The large standard deviations for some Semarkona and stratospheric dust data points are due largely to sample heterogeneity rather than low precision of the measurement. This observation is borne out by the fact that measurements on the three fragments of particle r21-M4-7 give distinct D/H values.

Several workers have shown that certain fractions (such as, acid insoluble residues) of several carbonaceous chondrites exhibit large, positive δD values³⁻⁶. Both Renazzo and Semarkona are unusual in that bulk samples of the meteorites exhibit large deuterium anomalies⁷. Deuterium excesses have been shown to be inversely correlated with increasing metamorphic grade in Type 3 chondrites⁸. Other chondrites have also been shown to exhibit deuterium anomalies⁹. In the first reported ion-probe measurements of D/H ratios in extraterrestrial materials, Hinton *et al.*¹⁰ obtained δD values ranging from 450 to 4,000 (average, 2,200) in five small spot scans ($15 \mu\text{m}^2$) of Semarkona. Our results on Semarkona confirm the large heterogeneity of the D/H composition on a small spatial scale.

Previous deuterium measurements in whole rock Renazzo samples weighing ~ 100 mg have given δD values ranging from -28 to $+1400$ in different temperature fractions measured in stepwise pyrolysis⁵. Note that our Renazzo data show both large positive and negative anomalies.

Particles r21-M3-5 and r21-M4-7 were found on impact collector W7021 provided by the cosmic dust collection group at the Johnson Space Center¹¹. This collector was flown on a NASA WB57F aircraft from 7 July to 15 September 1981 and had a total collection time at altitude ($\geq 60,000$ ft) of 44 h. Various particles were handpicked from the silicone oil coating on the collector. Each particle was transferred to a Nuclepore mount and washed in xylene for 2 min to remove the silicone oil¹². Measurements on untreated and xylene-treated Murchison matrix demonstrated that washing in xylene does not change the original D/H ratio of carbonaceous material.

Initial scanning electron microscopy (SEM) characterization showed that particle r21-M3-5 consisted of two apparently related fragments designated r21-M3-5A and r21-M3-5B. The former was subsequently transferred to a quartz plate, crushed, and then imbedded in the surface of a KBr crystal for IR transmission measurements. Two pieces of the second fragment, r21-M3-5B α and 5B β , were pressed in a Au foil for ion probe measurements. Particle r21-M4-7 broke into three fragments designated 7 α , 7 β and 7 γ , during mounting on the Au foil.

In Fig. 1 we show SEM photographs of r21-M3-5B and r21-M4-7. The energy-dispersive X-ray (EDAX) spectra seen in Fig. 2 show that the particles fall into the 'chondritic' subset of stratospheric dust particles¹³.

The question then arises whether the two stratospheric dust particles existed as small objects in space or whether they are fragments produced during atmospheric entry of larger, more familiar objects such as carbonaceous or ordinary chondrites three of which are known to exhibit large bulk D/H ratios. Although this possibility cannot be ruled out for any one particle, we consider it unlikely. Taken as a class, the chondritic

Table 1 Experimental results

	δD^*	$\sigma^\dagger$
Basalt glass KK-12-40‡		
Grain no. 1	+30.4	± 18.0
2	-46.8	± 16.1
3	+36.8	± 12.6
Basalt glass CD11-5§		
Grain no. 1	-79.7	± 9.3
2	-133.8	± 11.8
4	-111.1	± 17.2
5	-15.8	± 20.4
6	-110.4	± 43.4
7	-94.5	± 33.7
15	-123.7	± 14.5
16	-98.7	± 24.0
17	-128.1	± 36.9
18	-79.9	± 39.4
Actinolite		
Grain no. 1	+0.3	± 6.5
41	+73.8	± 32.2
42	+19.9	± 35.9
43	-112.2	± 31.6
51	-58.9	± 13.4
52	+27.5	± 13.3
53	+28.3	± 17.4
101	-81.9	± 17.4
102	-83.9	± 15.6
103	-100.4	± 24.9
104	-88.5	± 31.5
105	-65.2	± 15.2
106	-77.5	± 20.2
107	-67.5	± 21.6
108	-76.3	± 13.0
110	-119.4	± 17.7
111	-109.8	± 13.0
112	-55.4	± 17.0
113	-56.9	± 21.3
450	-113.8	± 22.2
452	-60.9	± 80.2
Renazzo		
Grain no. 1	-506.9	± 35.7
2	-71.2	± 41.4
3	-29.5	± 26.7
4	+529.4	± 49.2
Semarkona		
S0	+763.4	± 301.6
S1	+114.5	± 47.1
S2	+1,816.9	± 40.8
S3	+44.0	± 25.9
S4	+186.7	± 103.2
S5	+506.3	± 100.7
S6	+3,474.1	± 226.0
S7	+649.4	± 184.0
S8	+2,861.2	± 54.2
S9	+1,939.6	± 42.3
S10	+661.3	± 31.3
Stratospheric particles		
r21-M3-5B α	+818.5	± 92.6
r21-M3-5B β	+625.3	± 54.2
r21-M4-7 α	+615.9	± 235.5
r21-M4-7 β	+497.7	± 163.1
r21-M4-7 γ	+1,133.0	± 50.2

* δD values are relative to the SMOW D/H ratio of 0.00015576.

† Standard deviations of repeated measurements. Each measurement consists of between 30 and 50 peak jumping cycles.

‡ H₂O concentration is 0.11 wt%

§ H₂O concentration is ~ 1 wt%.

subset of stratospheric dust particles has been shown to consist of unique forms of extraterrestrial material. The internal morphologies and mineral assemblages, as measured by transmission electron microscopy and electron diffraction, differ from those found in meteorites¹⁴⁻¹⁶, the densities of individual particles are frequently low¹⁷, they contain noble gases characteristic of solar wind implantation^{18,19}, and their IR spectra fall into three distinct categories, two of which exhibit spectra different than those measured for carbonaceous chondrites²⁰. The large sulphur concentration of both analysed particles indicates that they have probably not been severely heated¹. They do not exhibit the 'droplet' appearance that would be expected for a product of atmospheric ablation and obviously did not melt²¹. However, more measurements will be required to verify the existence of the D/H enhancement in a broader range of particles including those that are demonstrably non-meteoritic.

The IR transmittance spectrum of r21-M3-5A falls into what has been termed the hydrated-silicate class III category. This class is well represented in the 21 stratospheric dust particles that have so far been measured²⁰. However, the spectrum is similar to that seen in a sample of Murchison²² and thus cannot be used to prove that r21-M3-5 is not a fragment produced during atmospheric entry of a carbonaceous chondrite. No IR measurements were made on r21-M4-7.

Although it is beyond the scope of this letter to discuss the possible cometary origin of most interplanetary dust particles, all the work done to date, including comparison of laboratory IR spectra with astronomical observations of comets²⁰, is con-

sistent with this hypothesis¹. Our observations suggest that comets may be 'primitive' objects, as is generally believed²³.

Large D/H ratios probably reflect the chemistry of the formation of hydrogen-bearing compounds and are unlikely to have a nuclear origin²⁴. Whether this chemistry took place in the early solar nebula or whether the dust grains (or some part of them) predate the formation of the Solar System and are relict interstellar grains²⁵ remains an open question. (Some authors have interpreted previous measurements of large D/H ratios in meteoritic materials as being caused by the formation of polymeric material in interstellar gas-dust clouds which are known to possess D/H ratios ~100 times higher than the standard terrestrial value. This interpretation is, for example, given in ref. 5.)

In this regard, the previously published evidence by Esat *et al.*²⁶ for statistically-significant $\delta^{26}\text{Mg}$ anomalies in several chondritic aggregates is interesting. By pushing conventional mass-spectrometric techniques to new levels of sensitivity these authors found effects at the level of 3–4%. Although the results are "not clearly established", they indicate that other isotopic anomalies, including some of possible nucleosynthetic origin, may be found through further careful work.

The variation of D/H ratios from one 10- μm fragment to another in Renazzo and Semarkona (and we suspect in many other objects), suggests that detailed study of such fragments by microanalytic techniques may help to identify the carriers of the deuterium enrichments.

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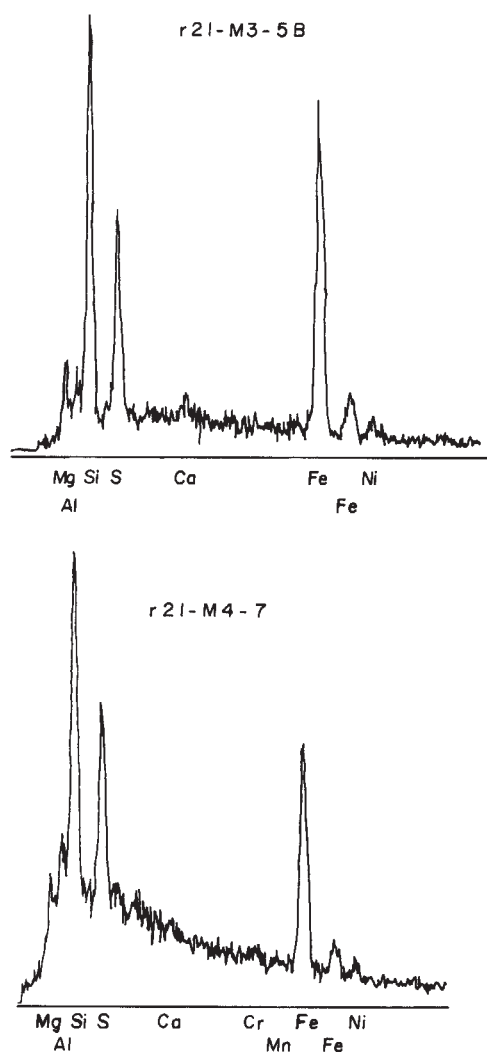


Fig. 2 EDAX spectra of particles r21-M3-5B and r21-M4-7.

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Dispersion limitations of oxidation in power plant plumes during long-range transport

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During the atmospheric transport of combustion-generated emissions of SO_2 and NO from point sources, the kinetics of oxidation processes are of particular importance in determining the pattern of subsequent wet and dry deposition because oxidation products such as sulphate aerosol and nitric acid may have deposition characteristics totally different from those of primary emissions. Modelling studies¹ have indicated the importance of the ambient air composition and of the dispersion rate on chemistry within plumes during the early stages of transport from point sources, and the important role of mixing in determining oxidant concentrations, particularly ozone, has been demonstrated in near field measurement programmes²⁻⁵. It has been generally assumed (see ref. 5) that the significant consequences of dispersion are confined to the early stages of long-range transport and observations to date (see refs 5, 6) indicate that the strong influence of dispersion on plume chemistry is exerted over distances <100 km from source. We report here results of measurements made by an instrumented aircraft of the chemistry within a labelled plume on a trajectory over the North Sea which indicate that oxidation of plume material may be limited by dispersion at distances of at least 650 km.

The measurements, which formed part of a much larger programme⁷, were made on 28 and 29 January 1981 using the Lockheed C130 aircraft of the Meteorological Research Flight. This aircraft has been equipped with modified commercial instruments for the continuous measurement of O_3 , NO_x and SO_2 , together with a specially developed electron capture detector for the real-time measurement of sulphur hexafluoride, the tracer used to unambiguously identify the power plant emission.

During the measurement exercise, emissions were transported in a northeasterly direction and wind speeds were in the range $5\text{--}10\text{ m s}^{-1}$. The mixing layer was $\sim 400\text{ m}$ deep (determined from temperature, dew point, and liquid water content profiles) and filled with stratiform cloud with a liquid water content $0.2\text{--}0.6\text{ g m}^{-3}$ and a mean volume droplet diameter $\sim 6\text{ }\mu\text{m}$. Insolation levels in the mixing layer were $\sim 15\%$ of the maximum values expected for clear air at the times the measurements were made.

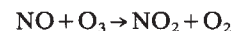
Sulphur hexafluoride tracer was injected into the plume from Eggborough Power Station (lat. $53^\circ 42'\text{ N}$, long. $1^\circ 6'\text{ W}$) and the labelled plume also contained the emissions from two nearby power plants after a short travel time. This is a common occurrence for most trajectories carrying emissions over the North Sea. Ground-based measurements indicated that plume material was fully mixed vertically within the boundary layer within $\sim 20\text{ km}$ of the sources for daytime releases.

The labelled plume was intercepted off the north-east coast of the UK $\sim 105\text{ km}$ from source on 28 January, corresponding to a release time of $\sim 0900\text{ GMT}$ and a travel time of $\sim 5\text{ h}$. It was intercepted again on 29 January near to the Danish coast, $\sim 650\text{ km}$ from source, corresponding to a release time of $\sim 1200\text{ GMT}$ on 28 January and a travel time of $\sim 24\text{ h}$.

Figure 1 shows a typical plot of the $[\text{NO}_2]/[\text{NO}]$ ratio and the ozone concentration near the centre of the mixing layer at 105 km , and Fig. 2 shows similar plots at 650 km . The tracer clearly identifies the position of the Eggborough Power Station plume. NO_x concentrations at the plume centre were 70 p.p.b.

(parts per 10^9) at 105 km and 35 p.p.b. at 650 km . Further south, changes in both $[\text{O}_3]$ and $[\text{NO}_2]/[\text{NO}]$ are associated with sources in the Trent Valley.

During the early stages of transport the chemistry of nitric oxide is dominated by reaction with ozone mixed into the plume from the ambient air²⁻⁴



and, because this reaction is rapid compared with the rate of mixing, plumes are characterized by an ozone deficit with respect to the background air. The measurements show that on this occasion less than half of the primary NO had been oxidized throughout much of the plume and less than one-third in the plume centre even after $\sim 24\text{ h}$ plume travel. In low insolation conditions, little ozone generation from entrained precursors is expected and the higher NO_2/NO ratio at the edges of the plume is clear evidence that the rate of oxidation was being determined by the rate of mixing of O_3 from the ambient air outside the plume. Indeed, the O_3 concentration along the centre line of the plume was depleted to virtually zero on both days, rising to a level of $\sim 20\text{ p.p.b.}$ in the air further north which is around the value normally observed for clear air at this latitude in mid-winter.

Previous measurements²⁻⁶ have mainly been restricted to the near field ($d < 100\text{ km}$) due to the lack of unambiguous plume identification and for logistical reasons, earlier studies were performed over land in clear air. In such conditions it is generally found that for daytime emissions, substantial plume dispersion occurs and that $>50\%$ of the plume NO is oxidized within $2\text{--}4\text{ h}$. Our own measurements in similar conditions show substantial agreement with this finding. However, a preliminary analysis of the results from several measurements over the North Sea (B. Callendar, personal communication) suggests that lower dispersion rates may be common for wind trajectories carrying UK emissions to Europe and hence that 'early' stages of plume development for a vertically mixed plume can cover much longer times and greater travel distances than previously measured²⁻⁶.

Major reactive radical oxidants in the atmosphere are produced by rapid complex reaction sequences involving ozone^{1,5-8}. Their concentrations are closely related to local ozone concentrations^{5,6}, and would be correspondingly low within slowly dispersing plumes. This has important implications for the oxidation of SO_2 . The major gas-phase oxidation route involves the hydroxyl radical^{1,5-8} and calculations using a gas phase

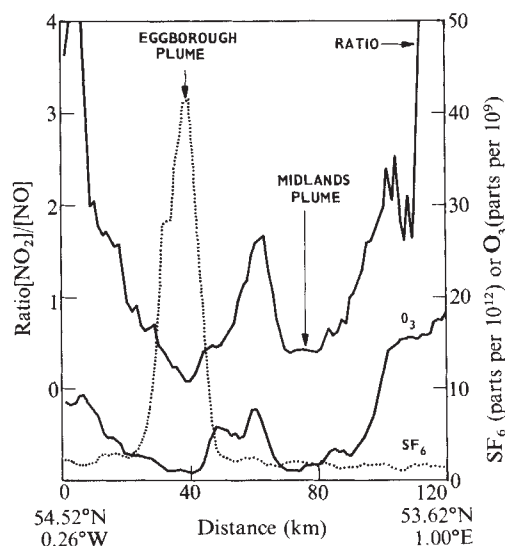


Fig. 1 Cross-plume profile, 28 January 1981. Height, 150 m . Distance from Eggborough Power Station, 105 km (latitude and longitude in decimal degrees).

chemical model described previously⁸ show that the ratio $[\text{OH}]/[\text{O}_3]$ is likely to be $<5 \times 10^{-6}$ in all daylight conditions and considerably less overnight. Taking $[\text{O}_3] = 2$ p.p.b. near the plume centre, the maximum $[\text{OH}]$ is thus calculated to be 10^{-5} p.p.b., which gives a maximum effective first order rate coefficient for gas phase oxidation of SO_2 of $\sim 0.1\% \text{ h}^{-1}$. In contrast, in the ambient clear air with $\text{O}_3 \approx 20$ p.p.b., the maximum rate constant would be $\sim 1\% \text{ h}^{-1}$.

Major routes for the oxidation of SO_2 in cloud droplets are expected to involve ozone or hydrogen peroxide⁹. The former reaction would not be important within the plume in conditions similar to those observed during the measurement exercise as ozone levels were very low. In low insolation conditions, hydrogen peroxide is a relatively stable oxidant accumulated as a result of slow overall gas-phase chemical processes. Virtually unmodified ambient concentrations could thus be entrained by the plume. Kinetic modelling studies, to be reported elsewhere, indicate that the ratio $[\text{H}_2\text{O}_2]/[\text{O}_3]$ does not exceed $\sim 3 \times 10^{-2}$ in a wide range of atmospheric conditions. Hence, in conditions similar to those of 28 and 29 January 1981, $[\text{H}_2\text{O}_2]$ is unlikely to exceed 0.7 p.p.b. outside the plume area.

Taking the amount of NO oxidized as a measure of the amount of O_3 entrained into the plume, if half of the total NO is oxidized it follows that the mass of H_2O_2 entrained (in molar units) is probably $<3 \times 10^{-2} \times 1/2$ (moles NO_x in plume). In the initial emissions, $[\text{SO}_2]/[\text{NO}_x] \approx 3$ and this ratio is approximately maintained at 24 h. Hence, the maximum amount of H_2O_2 entrained is only 0.5% of the plume SO_2 after 24 h, yielding a conversion rate of about $0.02\% \text{ h}^{-1}$ assuming a simple stoichiometric reaction between SO_2 and H_2O_2 .

The low production of OH and other oxidizing radicals within the plume, would also result in negligible SO_2 oxidation rates through the recently postulated mechanism involving radical scavenging by droplets¹⁰.

These calculations suggest that no significant oxidation of plume SO_2 to sulphate would occur in the condition studied in a period of 24 h. This conclusion is supported by measurements and detailed modelling studies to be reported elsewhere.

Thus, if conditions for slow plume dilution occur reasonably frequently, dispersion limitation of oxidant availability and consequent low effective rate constants for oxidation of plume materials may seriously affect the validity of large-scale predictive models using ambient air values for SO_2 and NO_x transformation rates.

In conditions of high insolation, photochemical reactions within a plume complicate the simple oxidant ingress picture but for low dispersion, limitations on the ingress of oxidant and

precursors may still substantially reduce in-plume oxidation processes.

We are indebted to many colleagues at the Meteorological Office, the Meteorological Research Flight and the Central Electricity Research Laboratories who participated in this programme, which was partly funded by the Electric Power Research Institute (Contract RP 1311-1). This work was carried out at the Central Electricity Research Laboratories and is published by permission of the Central Electricity Generating Board.

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Timing, trace and origin of basaltic migration in eastern Australia

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Eastern Australian Cenozoic volcanism includes a prominent southward migration of activity. Varied explanations have invoked hotspot or linear magma sources in the asthenosphere, the movement of Australia over old seafloor spreading zones—with direct or delayed response in volcanism, or changes in stress fields. The present appraisal, using new data, contradicts some ideas and favours volcanism triggered directly by passage of lithosphere over initial sites of Coral Sea spreading. The conclusions suggest that eastern Australia has a major, complex hotspot trail established for the past 65 Myr. It forms an independent and key continental reference to the Hawaiian-Emperor oceanic trail. It raises the notion that both continental and oceanic trails can mark the ghosts of former spreading sites.

Hotspots, their relation to volcanic chains and application as reference frames for plate motions have generated an extensive and partly controversial literature¹⁻⁶. They are important in studies of lower crustal and mantle evolution, both below ocean floors⁷ and in underplating continental and geomorphic processes⁸. They have implications for geophysical and mathematical modelling of the Earth's thermal regime^{9,10} and bear on the search for economic mineral deposits¹¹ and understanding of biological distributions¹². Most hotspot trails are largely oceanic features¹³ and proposed continental trails are generally unsubstantiated¹⁴ or relatively limited in extent¹⁵. An exception is the eastern Australian basaltic migration which traverses at least 2,000 km of continental crust^{16,17}.

In a previous study, I related major Australian volcanic centres to movement of the continent over former spreading anomalies for the Coral Sea system¹⁷. Smith⁸ also used motion over old spreading ridges to study continental responses, but his model based on African evidence favoured a thermal means that introduced delays of 6-30 Myr for volcanism and up to 60 Myr for uplift in eastern Australia. Pilger¹⁵ examined the trace of the Australian centres relative to the Hawaiian hotspot reference frame, but dismissed them as a true hotspot trace. He interpreted them as successive cessations in volcanism resulting from a change in stress field. These conflicts are discussed from further age dating of Australian volcanism

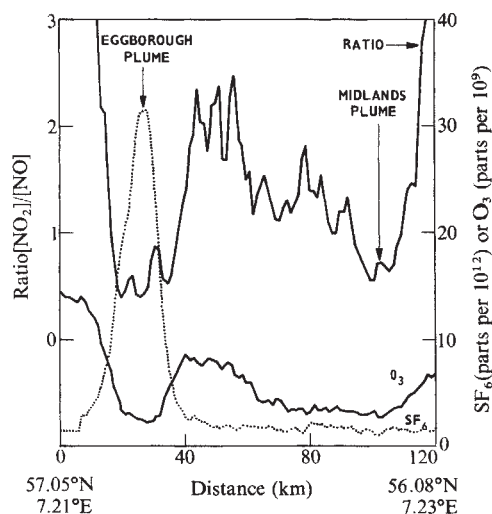


Fig. 2 Cross-plume profile, 29 January 1981. Height, 150 m. Distance from Eggborough Power Station, 650 km (latitude and longitude in decimal degrees).

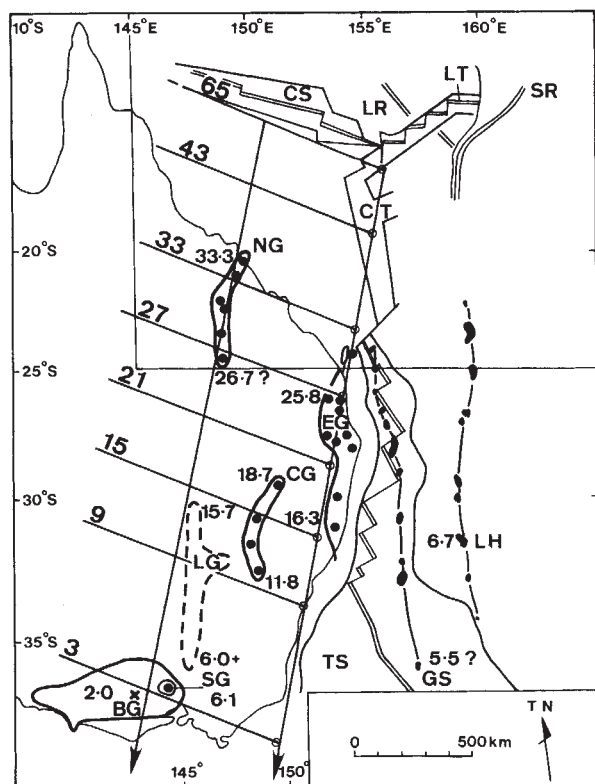


Fig. 1 Distribution of major migratory volcanoes and related volcanic fields, Eastern Australia and Tasman Sea in relation to seafloor spreading zones. Volcanoes with felsic culminations are shown as dots within enclosed groups (NG, Northern Group; EG, Eastern Group; CG, Central Southern Group; SG, Southern Group), with leucite lava field (dashed enclosure, LG), younger basalt field of Victoria (enclosure, BG, with mean position as cross) and Tasman seamount chains (irregular blobs, LH, Lord Howe Island; GS, Gascogne Seamount). Ages of northerly and southerly extents of groups are indicated by small numbers (Myr). Seafloor spreading features are shown as basins, with spreading ridges (double lines) joined by transform faults. (CS, Coral Sea Basin; LT, Louiade Trough; CT, Cato Trough; LR, Louiade Rise; SR, South Rennell Trench; TS, Tasman Sea Basin). Relative age positions (larger figures, Myr) of the southern margin of the Coral Sea Basin and Louiade Trough–Cato Trough triple point to Australia's migration⁸ (lines connected to small circle) are based on revised Southern Ocean spreading rates ($90\text{--}43$, 9 mm yr^{-1} ; $43\text{--}20$, 53 mm yr^{-1} ; $20\text{--}10$, 51 mm yr^{-1} ; $10\text{--}0$, $<73\text{ mm yr}^{-1}$) and a migration direction of 16° SSW (arrowed lines). Note close correspondence between successive positions of the Coral Sea margins with onset of basaltic volcanism (within 1 Myr). Box shows inset location for the more detailed Fig. 2.

(F.L.S. and P. Wellman, unpublished K–Ar dating), a revision of the spreading history in the Southern Ocean¹⁸ and recent refinement of the Australian migratory trace¹⁹. This study is based on the hotspot reference frame as the most appropriate frame fixed to the mantle, which may not apply to other frames such as polar wander curves⁴.

In my earlier study, a 53-Myr breakup was used for the Australian–Antarctic opening to control the migration of Australia relative to the volcanism¹⁷. This projected a broad trail from the Coral Sea spreading system through the general periods of basaltic activity of the main volcanoes. However, extrapolation of migration rates of the culminating felsic activity of the volcanoes projected back beyond the Coral Sea episode.

In the new analysis, the migration trends of the successive volcanoes are extrapolated back to the time of spreading of the Coral Sea–North Tasman Sea basins (>63 to 56 Myr)^{20–22}. The volcanic centres form several geographical groups (Fig. 1) and the projections (Table 1) use (1) mean age positions of these groups for the culminating activity, standardized for new age

dating constants; (2) migration of Australia at revised Southern Ocean spreading rates¹⁸; (3) for comparison, migration at a constant rate of lithospheric rotation over a linear source ($5.4^\circ\text{ Myr}^{-1}$)¹⁹. This linear trace is consistent with the orientation of the Coral Sea spreading axis and has a migration path agreeing with the volcano ages to within their experimental error.

Linear regressions taken through the volcanic groups show the highest regression coefficient ($R = 0.981$) for the northern group. This trend ($S16^\circ\text{W}$) is close to the average of basaltic trends that I found¹⁷ and given by Smith's reference frame⁸. It is used to project mean ages of the volcanic groups to positions relative to the Coral Sea spreading episode (Table 1, Fig. 2).

Both the 56 and 63 Myr positions are shown in Fig. 2, but those at 63 Myr lie close to the southern margin of the spreading system, with the main eastern group extending across the triple point position. The eastern group includes the most voluminous centres²³, but also more restricted younger and older groups which project on the lesser arms of the spreading system along the Louiade and Cato Troughs respectively. If a suggested slightly earlier spreading is taken at 65 Myr (ref. 22), the plots using a constant rate of linear rotation lie within $0.1\text{--}1.0^\circ$ and mostly between 0.1 and 0.2° lat. to the 63-Myr Southern Ocean rate positions. Thus, both methods give broad correspondence with the Coral Sea spreading margin and smaller angles of projection¹⁶ barely shift plots across the axis of the structure. Considering the variables involved, this strongly suggests volcanic trails originating at an initial spreading rift. They probably mark zones of high heat flow analogous to, but younger than, those identified along the eastern Australian margin. There, rocks show thermal overprinting of up to 200°C in the late Cretaceous, as a prelude to southern Tasman Sea spreading^{24,25}.

The new projections match volcanism directly to migration position over an activating spreading feature (Figs 1, 2). Delays in volcanism inherent in Smith's projection, with a 55-Myr Australia–Antarctic breakup largely disappear. His suggested delays in plateau uplift also need reconsideration as relationships between volcanism and uplift are complex^{26,27} and probably include at least some contemporaneous uplift¹⁹. The precise mechanism by which volcanism is triggered as lithosphere over-rides spreading zones remains unclear, but residual heating, underplating, metamorphism, metasomatism, degassing or uplift all need evaluation^{26–30}. Extensive volcanism in eastern Australia, older than the main migrational trail described here (Fig. 3) may also include migrational trails^{17,19}. A detailed analysis of the earlier Tertiary volcanism for possible migration relationships to the older Tasman spreading margin is in preparation³¹.

Pilger's rejection of a hotspot trace for eastern Australia¹⁵ is also suspect. Previously¹⁷, I incorporated leucite and basalt lava fields in the trail, which would modify the distribution depicted by Pilger to closer alignment with the Hawaiian reference trace. The new data support this¹⁹ and show the leucites project on the Coral Sea margin at 63–65 Myr (Table 1, Fig. 2). Discrepancies in the Hawaiian hotspot trace referred to Australia may reflect uncertainties in mutual positions of the Australian, Antarctic and Pacific plates since the late Cretaceous³². Certainly, the cessation of volcanism Pilger proposed along the Australian chain on incomplete 1974 data does not hold on more complete dating^{17,23,33,34} (Fig. 3).

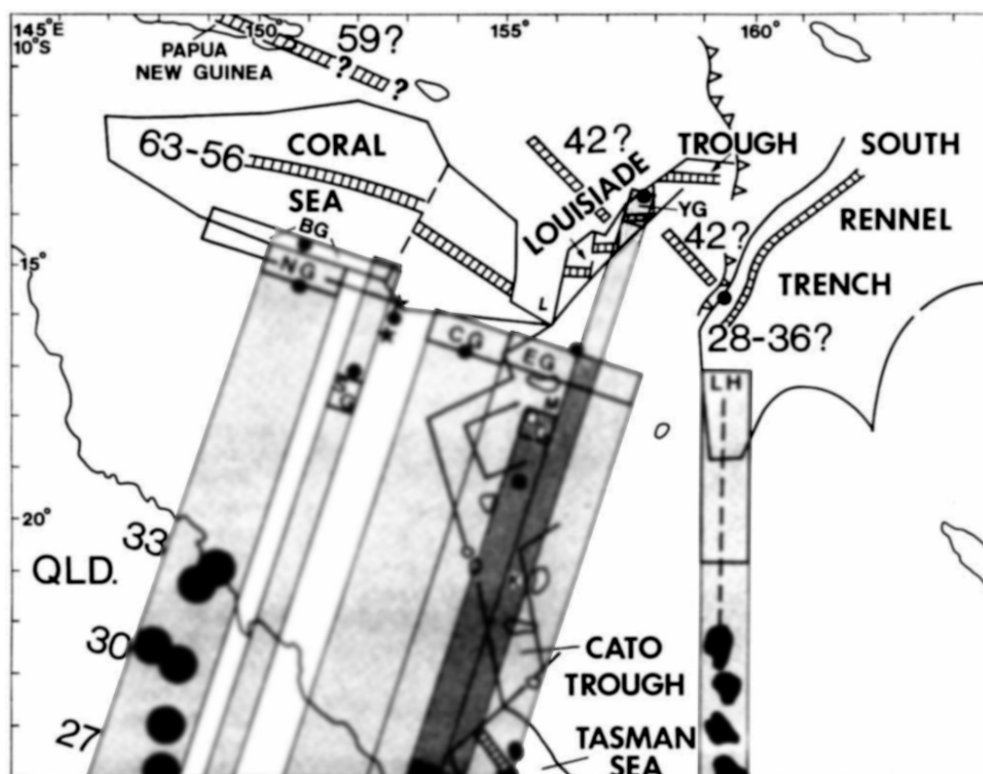
Migratory seamount chains on the Tasman sea floor^{35–37} were also compared by Pilger with the Hawaiian reference trace. The trace is 7 Myr too old for Lord Howe Island which may reflect a separate age and origin for the eastern and western chains. The eastern Lord Howe chain projects north towards an Oligocene sea floor along South Rennell Trench³⁸, tentatively correlated with anomalies 8 and 9 and possibly with anomaly 12 (28–36°); it projects on the postulated spreading centre at 36 Myr (Table 1, Fig. 2). The asymmetrical spreading here is similar to the South Fiji Basin, where western sea floors are lost at continental rises^{39–42}. If it is due to subduction, this requires anticlockwise rotation of the Lord Howe chain since

Table 1 Positions, ages and projected latitudes for volcanic groups plotted in relation to spreading zones in the Coral Sea region in Fig. 2

Volcanoes (Groups)	Long., Lat	Age (Myr)	Projected Lat.
Northern Group (Hillsborough-Buckland)	148–149° E, 21–25° S	33.3–26.7	15.4° S (56 Myr)
Cape Hillsborough	Mean 148.3° E, 22.7° S	Mean 30.3	14.8° S (63 Myr)
	149.0° E, 21.0° S	33.3	15.5° S (65 Myr)
Eastern Group (Cooroy-Comboyne)	152–154° E, 26–32° S	25.8–16.3	17.3° S (56 Myr)
Mt Cooroy	Mean 152.6° E, 28.4° S	Mean 22.3	16.7° S (63 Myr)
	153° E, 26.4° S	25.8	16.8° S (65 Myr)
Older Eastern Group (Fraser Island-Focal Peak)	152–154° E, 25–29° S	33?–23.0	18.4° S (56 Myr)
Fraser Island	Mean 152.6° E, 28.4° S	Mean 28?	17.8° S (63 Myr)
	153° E, 25° S	33?	19.4° S (65 Myr)
Younger Eastern Group (Mt Juillerat)	153° E, 28.4° S	16.2	14.1° S (56 Myr)
Mt Juillerat	153° E, 28.4° S	16.2	13.5° S (63 Myr)
	153° E, 28.4° S	16.2	13.6° S (65 Myr)
Central Southern Group (Nandewar-Orange)	148–151° E, 30–34° S	18.7–11.8	16.8° S (56 Myr)
Nandewar North	Mean 149.2° E, 31.4° S	Mean 15.5	16.2° S (63 Myr)
	150° E, 30.1° S	18.7	16.7° S (65 Myr)
Leucitite Group (Cobar-Cosgrove)	145–147° E, 31–37° S	15.7–6.0	16.4° S (56 Myr)
El Capitan	Min. 145.6° E, 36.3° S	Min. 6.0	15.9° S (63 Myr)
	146.2° E, 31.2° S	15.7	16.1° S (65 Myr)
Southern Group (Macedon)	144.7° E, 37.4° S	6.1	17.9° S (56 Myr)
Mt Macedon	144.7° E, 37.4° S	Mean 6.1	17.3° S (63 Myr)
	144.7° E, 37.4° S	6.1	17.1° S (65 Myr)
Younger Basalt Group (Western Victoria)	141.5–145° E, 36.5–38.5° S	4.5–0.5	15.4° S (56 Myr)
Western Victoria	Mean 143.8° E, 37.5° S	Mean 2.0	14.8° S (63 Myr)
	Mean 153.8° E, 37.5° S	Mean 2.0	15.0° S (65 Myr)
Lord Howe Island (East Tasman chain)	159.0–159.1° E,	6.9–6.4	20.9° S (28 Myr)
	31.5–31.6° S	Mean 6.7	17.1° S (36 Myr)
East Tasman chain	31.5–31.6° S	Mean 6.7	15.6° S (36 Myr)
Gascoygne Seamount (West Tasman chain)	156.0–156.7° E,	6–5?	21.2° S (36 Myr)
West Tasman chain	36.2–36.8° S	Mean 5.5?	17.8° S (43 Myr)
	Mean 36.5° S	Mean 5.5?	16.0° S (43 Myr)

Ages of volcanoes are compiled from the literature cited in ref. 17 and updated from ref. 23 and F.L.S. and P. Wellman, unpublished dating. K–Ar ages are corrected to new constants for direct comparison with recent correlation of the oceanic magnetic anomaly time scale^{49,50}. Projected latitudes are determined at the following migration rates for Southern Ocean spreading, 90–43 Myr (9 mm yr⁻¹),¹⁸ 43–20 Myr (53 mm yr⁻¹), 20–10 Myr (51 mm yr⁻¹), 10–0 Myr (<73 mm yr⁻¹)⁵¹. The last latitude in each group, gives the projected latitude using a linear rotation of the lithosphere of 5.4° Myr⁻¹ (ref. 19) for the period 0–43 Myr.

Fig. 2 Projections of major volcano groups, eastern Australian region, in relation to seafloor spreading episodes. Volcanic groups are projected from mean positions to latitudinal positions listed in Table 1, using the linear regression angle of 16° for the northern group (filled circles with ages in Myr). The projected groups (stippled bands) show 56 and 63 Myr positions (boxes) with group symbols as for Fig. 1, and including the Older Eastern Group (OG) and Younger Eastern Group (YG). The leucitite group plots as stars and the Younger Basalt Group of Victoria as an unstippled box BG. The eastern Tasman seamounts (filled irregular outlines) are projected northwards (dashed line) to 28 and 36 Myr positions (stippled band) from Lord Howe Island. The western seamounts project north through Kenn Reef (K) to Mellish Reef (M) to Louisiade Rise (L). Projections using a constant rate of lithospheric rotation (5.4° Myr⁻¹) for 43–0 Myr interval plot as dots (65 Myr position for Eastern Australia). Seafloor spreading ridges (segmented strips) show spreading age (Myr) and a suggested thrust boundary along the western margin of South Rennell Trench (SR)⁴⁵. The 63-Myr positions of the projections form the northern leading box lines of the stippled trails at the Coral Sea spreading margin.



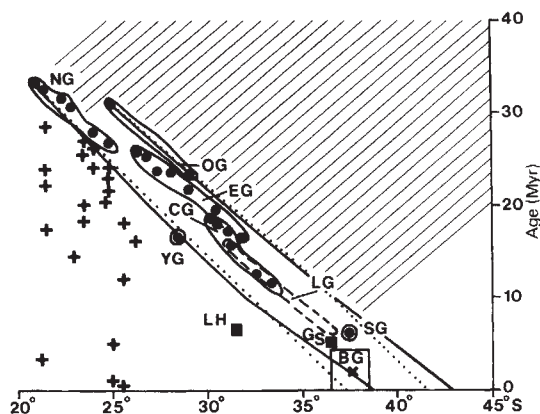


Fig. 3 Latitude-age plots of eastern Australian volcanism, showing volcanoes with felsic culminations, (group symbols as in previous figures). The region of older, continuous scattered episodes of basaltic activity falls in the shaded area. The trace drawn through the northern-most groups represents the rate of Southern Ocean seafloor spreading (solid lines) and constant rate of lithospheric rotation (dotted lines). Basaltic volcanism succeeding the migration of the major groups is shown by crosses (eastern Australia) and filled squares (Tasman seamounts). Dates are plotted from references cited in the text and from C.G. Murray (personal communication), P. J. Stephenson (personal communication) and F.L.S. and P. Wellman (work in preparation).

the Oligocene, as part of the tectonic realignments of the Australian plate boundary^{43,44}. Tentative projection of the western chain from subsidence data at Gascoyne Seamount³⁵ gives (Table 1) relatively older ages closer to the Hawaiian reference prediction¹⁵. At Kenn Reef, the 36-Myr plot is 4°S of that of the Lord Howe chain and isolated seamounts extend from here to Mellish Reef. The 43-Myr plot there is too young for the Coral Sea triple point, but coincides with possible spreading across Louisiade Rise at about 42 Myr (ref. 45) (Fig. 2). The 250-km migration of Louisiade Rise from 65 to 42 Myr places this axis close to Mellish Reef as a potential hotspot to initiate the western chain.

With the new amendments, the eastern Australian traces reasonably match the Hawaiian reference trace. However, the Macedon trachytes at 6 Myr are older than the predicted 2-Myr age and at 65 Myr project 2°S of the Coral Sea margin; instead the younger basalts of Victoria better match the Hawaiian trace and plot on the Coral Sea margin (Table 1, Fig. 2). This makes the Macedon trachytes early fractionates of K-rich basaltic and leucititic magmas and tholeiitic outpourings of the younger basalts the main volcanism over the Coral Sea hotspot. The broad pattern of the volcanic migration may represent a slowly dying hotspot, as suggested for the Caroline Islands⁴⁶. From the north end, voluminous trachytes and rhyolites²³ dwindle in amount to the south end, where trachytes and leucitites from lesser melting pass into purely basaltic activity¹⁷.

In conclusion, eastern Australian volcanism seems to project back to old spreading rifts of different size and age. These probably marked thermal zones which produced volcanic trails with the movement of the Australian plate over the spreading sites. If this is a general mechanism further examples of continental and associated oceanic trails should be present on moving plates. The migratory centres of southern New Zealand and Campbell Plateau⁴⁷ resemble the eastern Australian chains. Their relationships to the South Fiji Basin spreading zones within the complex tectonics of the plate boundary in New Zealand and the relationships of other hotspot traces to old spreading sites (Hawaiian and Caroline Islands) is under study⁴⁸.

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Evidence from north-west Canada for an early Holocene Milankovitch thermal maximum

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According to the Milankovitch theory of global climatic change, maximum summer solar radiation at high latitudes of the Northern Hemisphere occurred at 10,000 yr BP (refs 1, 2). In particular, it predicts summer solstice radiation greater by 9–10%. Preliminary climate simulation experiments with these increased values of radiation confirm that high-latitude land surfaces received maximal insolation at ~10,000 yr (refs 3, 4). Paradoxically, however, the large volume of fossil pollen and other evidence from North America indicates a maximum of Holocene warmth at 7,000–6,000 yr (ref. 5), and a recent review of the evidence from New England suggests that the warming began at 9,000 and ended at 5,000 yr, but also stresses the difficulties of interpretation in terms of climate change⁶. We summarize here data from sites in the north-west corner of mainland Canada (Fig. 1) that directly support the Milankovitch hypothesis.

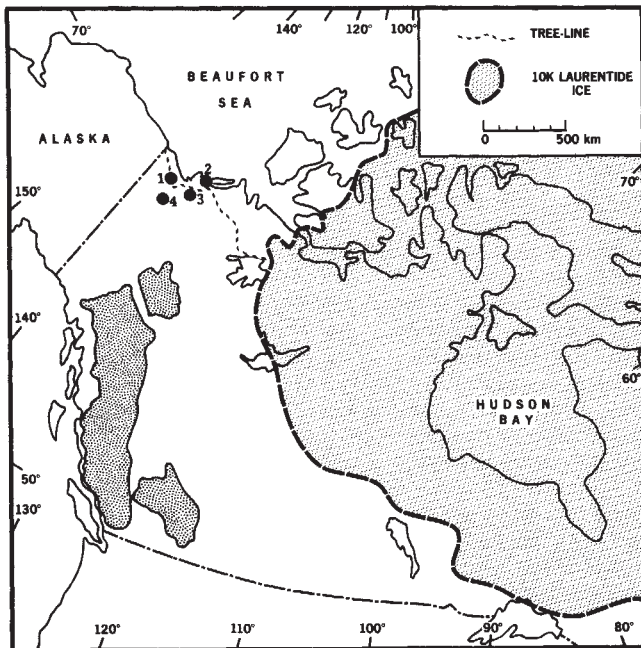


Fig. 1 Western and central Canada showing the site locations: 1, Hanging Lake; 2, Sleet Lake; 3, Twin Tamarack Lake; 4, Lateral Pond. Dashed line indicates the approximate position of Laurentide ice at 10,000 yr; solid line indicates the Cordilleran ice at 10,000 yr.

The area, centred on the Mackenzie River delta, was freed of Laurentide ice by 14,000 yr (ref. 7) and land to the west of the delta, in the northern Yukon, remained ice-free throughout the latest glacial period⁸. The uplands east of the delta have only moderate relief so that vegetational response to climate change was probably not inhibited by orographic barriers, as appears to have been the case in adjacent northern Alaska⁹. The area is traversed by the arctic tree line and is characterized by steep gradients of such parameters of summer climate as mean July temperature, growing season duration, total degree days above 5 °C and the frequency of Pacific air masses¹⁰.

A pollen record from Hanging Lake, a site beyond the modern limit of trees (site 1, Fig. 1), strongly supports an early warm interval dating from 11,100 to 8,900 yr (ref. 11). Total pollen influx increased from <500 to >1,000 cm⁻² yr⁻¹ and some taxa had more northerly distributions. Within the northern boreal forest zone in the uplands immediately to the east of the Mackenzie delta (site 3, Fig. 1), lake sites yield a consistent record of pollen for the past 14,000 yr. The central features are illustrated by pollen data from one lake, Twin Tamarack (Fig. 2)—they are: an increase in total pollen influx at 10,000 yr from values <500 during the herb-tundra phase (14,000–10,000 yr) to >2,000 at 10,000 yr, and peaks of pollen influx of dwarf birch and poplar ~10,000, with declines in the curves for non-arboreal pollen types. These, and other minor changes, are interpreted¹⁰ as a transition from tundra to woodland in response to a major warming of climate. A similar transition from tundra to boreal forest was recorded at Lateral Pond in the Richardson Mountains (site 4, Fig. 1), with an increase in total pollen influx from <400 to >1,000 at ~10,000 yr (ref. 12).

A small lake (Sleet Lake), 70 km north of the modern tree line, in the shrub tundra zone of the Tuktoyaktuk Peninsula (site 2, Fig. 1) shows convincing pollen evidence for the early Holocene warm period¹³, corroborating the conclusions based on earlier, less detailed analyses from a nearby site¹⁴. In particular, spruce influx (Fig. 3) began to increase sharply just before 10,000 yr, reaching a maximum at 9,000 yr, and the local presence of spruce is substantiated at 9,500 yr by the

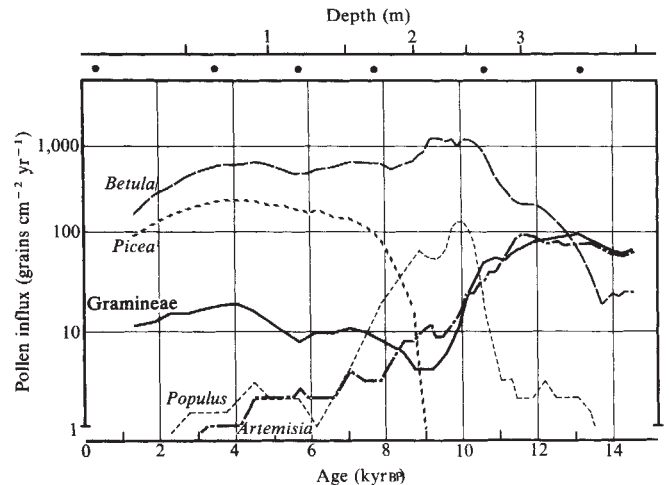


Fig. 2 Five sample running mean values of pollen influx (log scale) for grass (Gramineae), sagebrush (*Artemisia*), poplar (*Populus*), dwarf birch (*Betula*) and spruce (*Picea*), plotted against radiocarbon age before present and depth in the sediment. The site is Twin Tamarack Lake near Inuvik, North-west Territories. •, The position in the sediment of the radiocarbon age determinations; details are given in Table 1.

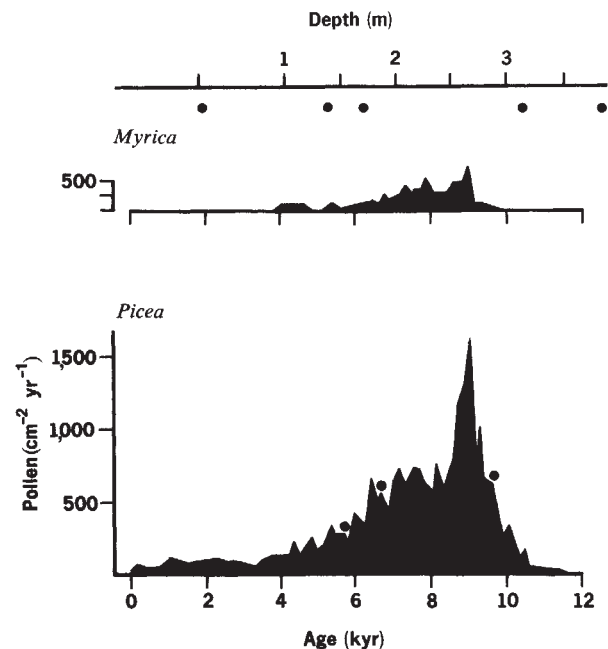


Fig. 3 Pollen influx values for *Picea* and *Myrica* (bog myrtle) plotted against radiocarbon age. •, Spruce needles. The site is Sleet Lake on the Tuktoyaktuk Peninsula, North-west Territories. The upper • indicate the positions in the sediment of the radiocarbon age determinations; details are given in Table 1.

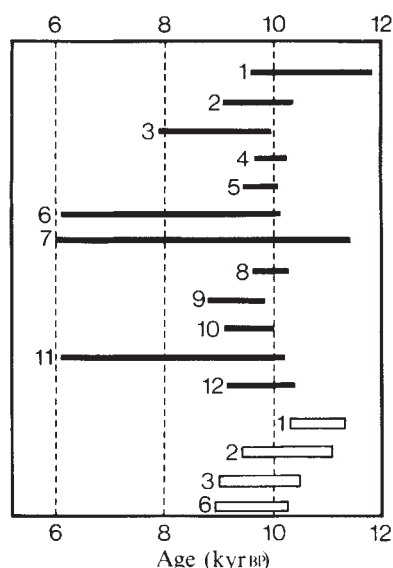
occurrence of spruce needles. The spruce record shows that between 10,000 and 6,000 yr much of the Tuktoyaktuk Peninsula, which is today covered by tundra, was then occupied by spruce forest and that by 4,000 yr the landscape had reverted to tundra. Summers during the earliest Holocene must have been significantly warmer than the modern Arctic and late Holocene climates (4,000 yr BP to present). This is the most convincing example of an early Holocene maximum tree line advance from anywhere in the Northern Hemisphere.

Table 1 Depth in the sediment and age of the dated samples from the Twin Tamarack (Fig. 2) and Sleet Lake (Fig. 3) sites

Site and location	Depth in sediment (cm)	Age* (yr BP)	Laboratory no.†
Twin Tamarack 68°18' N, 133°25' W	2–6	440 ± 60	GSC 3394
	67–73	3,640 ± 90	GSC 3377
	119–126	5,830 ± 90	GSC 3384
	163–172	7,810 ± 100	GSC 3347
	296–304	11,600 ± 140	GSC 3346
	320–330	13,100 ± 150	GSC 3387
Sleet Lake 69°17' N, 133°35' W	60–68	1,970 ± 60	GSC 3330
	113–120	5,270 ± 80	GSC 3323
	193–200	6,210 ± 110	GSC 3311
	302.5–310	10,400 ± 110	GSC 3307
	382.5–390	12,500 ± 110	GSC 3302

* In radiocarbon years before present.

† Radiocarbon Laboratory, Geological Survey of Canada, Ottawa.

**Fig. 4** Solid bars indicate the time span, in radiocarbon yr BP, within which *Typha* was recorded at the numbered sites; open bars indicate the range of peaks of poplar (*Populus*) pollen. The numbered sites are: 1, Hanging Lake¹¹; 2, M Lake¹⁷; 3, Twin Tamarack; 4, KE site Old Crow Flats²⁰; 5, BL site Old Crow Flats²⁰; 6, SW Lake¹⁰; 7, Sleet Lake¹³; 8, Mayday Lake (R.W.S., unpublished data); 9, Hendrickson Island pingo²¹; 10, Eskimo Lake pingo²⁰; 11, Richards Island pingo²²; 12, East Channel site (J.C.R., unpublished). The radiocarbon age of these sites is based on 62 sample analyses of organic lake sediment and bog peat. The details of sample depth, corrected age, error and laboratory number are given elsewhere¹⁰.

In addition to the above evidence, certain indicator taxa are restricted to the period about 10,000 yr. *Typha* (the cat-tail rush) is wind-dispersed over short distances¹⁵, so even isolated pollen grains in sediment indicate that the plant grew nearby. Its modern northern limit is several hundred kilometres to the south of our sites¹⁶ and we interpret *Typha* pollen in Holocene samples in terms of a climate warmer than today. It has been recorded from 12 sites (Fig. 4) and the occurrences cluster about 10,000 yr with a range of 6,000–11,800 yr. Poplar pollen peaks of 20–30% of total pollen per sample, similar to that at site 3 (Fig. 2), have been recorded at other sites and we include them in Fig. 4 to illustrate the clustering about 10,000 yr.

Finally, the boreal shrub, bog myrtle (*Myrica gale*), reaches its modern northern limit in the boreal forests of Mackenzie delta. However, Sleet Lake, a tundra site, yielded a *Myrica* pollen influx curve (Fig. 3) showing maximum occurrence beyond its modern range at ~9,000 yr and disappearance from that area by about 5,000 yr. Similar maxima and ranges of *Myrica* have been recorded at Hanging Lake¹¹, and in two unpublished¹⁰ and one published record¹⁷ near the Mackenzie delta. Poplar and *Typha* macrofossils in sediments from a site in western Alaska were used to support the first suggestion of an early Holocene warming¹⁸.

We conclude that the pollen and macrofossil evidence from the uplands surrounding the Mackenzie delta support the Milankovitch postulate of a period of maximum summer warmth centred on 10,000 yr. At least one set of independent data corroborates our findings: ¹⁴C-dated basal sediments of thermokarst basins along the coastal lowlands adjacent to the Mackenzie delta show a period of maximum melting of ground ice between 10,000 and 9,000 yr (ref. 18). Why, then, is the early Holocene net radiation maximum not an evident feature of most Northern Hemisphere palaeoecological reconstructions? From attempts to model past climates, it is clear that surface albedo is an important parameter that exerts a profound effect on regional and global climate dynamics¹⁹. Palaeoecological sites in central and eastern North America have probably not recorded an early Holocene pulse of warming because of the influence of Laurentide ice (Fig. 1) on atmospheric circulation patterns. In contrast, the Alaska–Yukon–Mackenzie delta area is upstream of the effects of the Laurentide ice and therefore sensitive to changes in net radiation. Furthermore, there is no evidence from anywhere in the Alaska–Yukon–Mackenzie delta area for a mid-Holocene (8,000–5,000 yr) thermal maximum. It was probably not until the Laurentide ice had significantly wasted away that the global temperature rose despite declining net radiation in high latitudes; this hypothesis needs to be tested using general circulation models.

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Continental margin origin for Cretaceous radiolarian cherts in Western Timor

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In Timor (Fig. 1) widespread outcrops of Cretaceous cherts belonging to the Wai Bua and Cretaceous Kolbano facies have been interpreted as deep-sea deposits that probably accumulated on the Australian continental rise¹. I consider here another but less well known group of Cretaceous cherts exposed in Western Timor at the base of the Palelo Group, which outcrops in mountain massifs with metamorphic rocks of the Mutis Complex. Although the observed contacts between the two units are faulted^{2,3}, the Palelo Group contains fragments of metamorphic rocks similar to those of the Mutis Complex^{4,5}, suggesting an original unconformable relationship. Geochemical data^{6,7} from metasedimentary gneisses in the Boi Massif suggest that metamorphism occurred at pressures and temperatures likely to prevail deep in the continental crust. I suggest that the Cretaceous radiolarian cherts were deposited on a continental margin as the first sediments of a forearc basin. This is the first reported occurrence of radiolarian cherts deposited in this tectonic-sedimentary environment, and contrasts strongly with other Cretaceous radiolarian cherts exposed on the island. The evidence is crucial to understanding the tectonic evolution of the region because it is clear that the two groups of cherts were derived from different sides of the continent-island arc collision zone which makes up the Banda Arcs. The Palelo Group and the rocks it is associated with were derived from the South-east Asian side, which has had an active margin since at least the early Cretaceous.

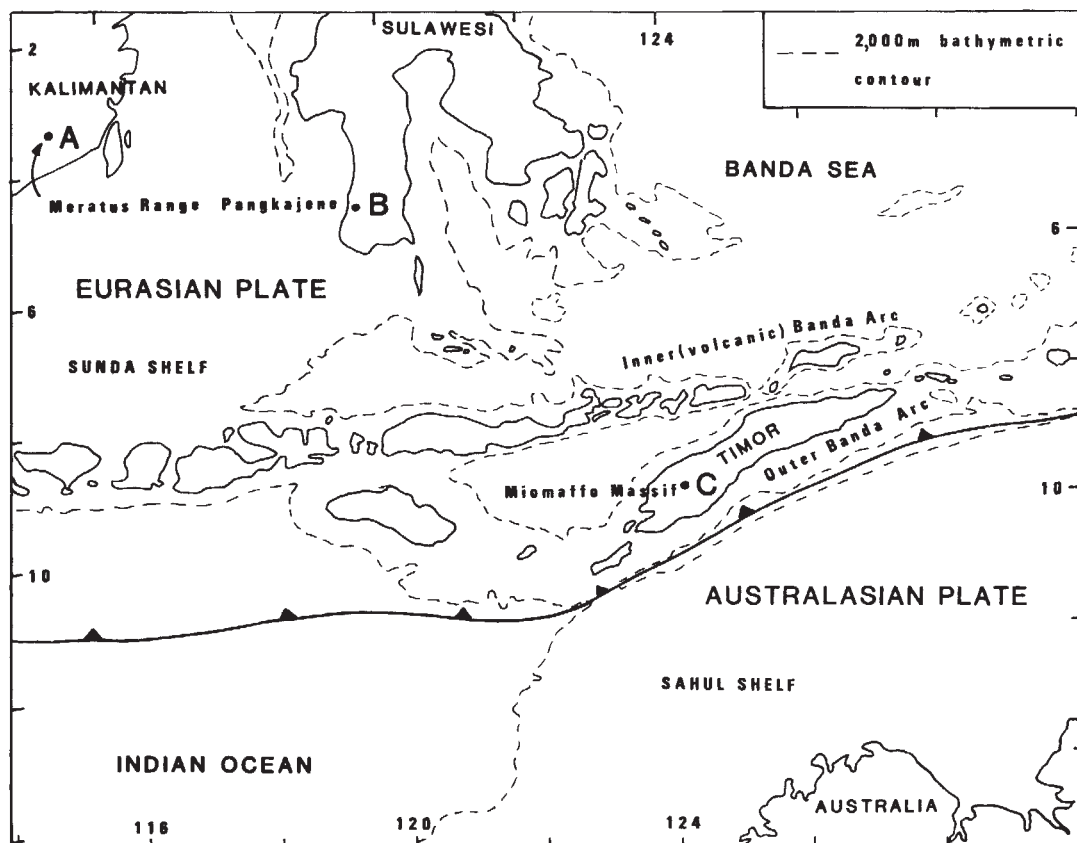
The general stratigraphy of the Palelo Group (Fig. 2) is well established from studies in the Miomaffo Massif^{3,5} (C in Fig. 1) where the most diverse assemblage yet observed is present. It consists of agglomerate and tuff overlain by bedded and colour-banded radiolarian chert with shale partings and interbedded sandstone, in turn overlain by limestone and chert, and succeeded by a unit of siltstone, sandstone and conglomerate, all of which contain andesitic debris.

In the Molo Massif the distinctively coloured radiolarian cherts of the Noni Formation are interbedded with tuffaceous clastics and andesitic volcanics^{2,4}, which are typical components of the overlying Haulasi Formation of the Palelo Group (Fig. 2). At least some of the cherts are tuffaceous. A sample (TM3057, Chelsea College, London) of brown-green chert collected from the River Oe Lelo contains angular and lath-shaped clasts of zoned and albitized plagioclase and clasts of colourless augite and pale green clin amphibole. Many of the fragments are derived from volcanic rocks, while some of the plagioclase and amphibole might have been eroded from basic gneisses of the underlying Mutis Complex. The presence of a contemporaneous source of clastic and volcanoclastic debris is the second piece of evidence that the cherts were deposited on or adjacent to continental crust.

The succession in the Cretaceous to Eocene sediments which overlie the Mutis Complex represents a shallowing upward sequence which starts with radiolarian chert and ends with nummulitic limestone and marl deposited in a neritic environment. Volcanic rocks and volcanic debris occur throughout the succession. Although their geochemical affinity has not been determined, the types of rocks present suggest derivation from a volcanic belt above a subduction zone⁸. Second, there is an upward trend from basic to intermediate to acid compositions, reflected in the change from basalt to andesite within the Palelo Group, and in the presence of ignimbritic tuff and dacite intercalated with Eocene carbonates in the Mosu Massif^{1,9}. It is evident that the sediments were deposited in a forearc basin floored by continental crust.

It is generally accepted that the Banda Arcs (Fig. 1) represent a collision zone between the passive continental margin of

Fig. 1 The geographical and tectonic location of Timor: A, B and C are localities mentioned in the text.



UNIT		LITHOLOGY	AGE
(un-named)		volcanics & nummulitic limestone & marl	EOCENE
PALELO GROUP	HAULASI FM	volcanics & tuffaceous clastics	PALAEOCENE
	NONI FM	limestone & radiolarian chert	LATE CRETACEOUS
		radiolarian chert, volcanics & tuffaceous clastics	
	METAN FM	agglomerate & tuff	?LATE JURASSIC
MUTIS COMPLEX		metamorphic rocks	PRE-LATE JURASSIC

Fig. 2 Stratigraphy of the Palelo Group of Western Timor.

Australia and an island arc on the fringe of South-east Asia. The most contentious and widely debated geological issue concerns the place of origin of different groups of rocks because this determines how the structure and tectonic evolution of the island and the entire region are interpreted. The conclusion that the Palelo Group was deposited in a forearc basin means that it and the underlying metamorphic rocks were part of South-east Asia before the collision occurred. This assertion is substantiated by the occurrence of similar and broadly contemporaneous successions in the Meratus Range of South Kalimantan^{2,3,5} and the Pangkajene region of south-west Sulawesi²⁻⁵, both on the Eurasian plate (Fig. 1A, B). Conversely, there are no comparable successions in northern Australia or beneath the Sahul Shelf, even though these areas are nearer to Timor than the two localities in Indonesia.

From the time of the earliest geological expeditions to Timor ~70 yr ago it has been recognized that Permo-Triassic rocks of two different facies exist side by side on the island. One explanation for this is that the different facies represent tectonic units from different plates that have been brought together by overthrusting^{1,10}, but an alternative model considers that all formations which predate the late Miocene to early Pliocene collision were derived from the Australian continent and were juxtaposed by normal faulting^{11,12}. For at least the Mutis Complex, Palelo Group, and overlying Eocene succession it has been shown that they were part of South-east Asia before the collision. By inference, therefore, the two contrasting facies of Cretaceous radiolarian cherts must have been brought together by overthrusting.

The wider implications of this work concern the origin and genesis of bedded radiolarian cherts, which often are regarded as pelagic deposits laid down on oceanic crust far removed from continental crust and terrestrial influences. Based on stratigraphical, lithological, and petrological information I suggested that the opposite is true for the Palelo cherts of Western Timor. Using geochemical data, it was recently proposed¹³ that late Triassic radiolarian cherts of central Japan were laid down on continental crust in an offshore or marginal marine environment, and other occurrences may come to light in the near future.

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Diatom evidence for recent acidification of two Scottish lochs

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Claims that highly acid ($\text{pH} < 5.0$), often fishless lakes have become so as a result of recent (mainly twentieth century) acidification processes have not been fully validated because there are few acid lakes that have been the subject of long-term continuous monitoring. However, diatom analysis of ²¹⁰Pb-dated sediment cores can provide the detailed historical perspective required. Here we use these techniques to show that some lakes in the UK have become strongly acidified within the past 130 yr. Analysis of cores from two lakes in Galloway, south-west Scotland, indicate that both lakes, one with an unaffected catchment and one with a recently afforested catchment, have experienced pH declines of approximately 1 pH unit (from ~pH 5.5 to 4.5) during this time.

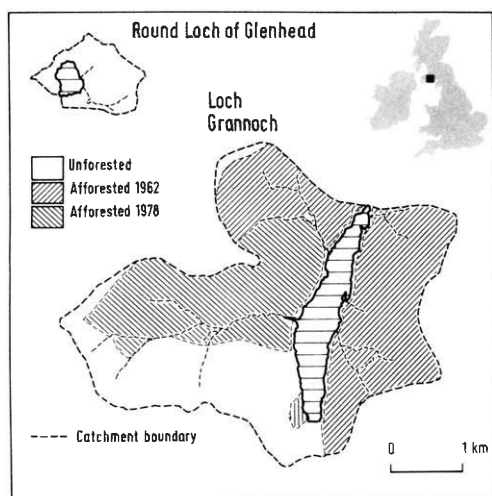


Fig. 1 Site location and lake catchment maps for Round Loch of Glenhead and Loch Grannoch showing areas afforested.

Following early reports of recent lake acidification in Norway¹, Sweden² and Canada³, lakes with pH values <5 have been noted in several other countries⁴⁻⁸. The high acidity of precipitation and the occurrence of acid lakes and streams in the UK have been documented⁹⁻¹⁵. There is dispute, however, as to whether these acid lakes and streams have become increasingly acid over recent decades. According to Sutcliffe *et al.*¹⁵ chemical records for some Lake District lakes show no evidence of acidification between 1928 and 1980. On the other hand, lakes draining granitic catchments in Galloway that currently have pH values ranging from ~4.5 to 6.0 (ref. 16), no longer support a salmonid fishery while others have declining fish populations¹⁷.

Increased acidity of lakes has been ascribed mainly to acid precipitation^{1-5,7,16,17}, but catchment land-use changes, including afforestation^{18,19}, and the longer-term continuous effects of soil leaching associated with early forest clearance and climatic change²⁰, have also been implicated. Because historical data are mainly inadequate, evidence for acidity changes in lakes can best be found in sediments by diatom analysis. Diatoms are siliceous algae and are usually found in great abundance and diversity in lakes and rivers. They are sensitive to changes in water quality and floristic analysis of their fossil remains in lake sediments can be used to reconstruct former pH values of lake water²¹⁻²⁴.

Our diatom analysis and ²¹⁰Pb dating of sediment cores from a range of lakes in the Galloway region not only assess the history of acidification in the area but also evaluate the role of afforestation as a cause of acidification. Lake and catchment characteristics of our comparison between a core from a loch with an unafforested catchment (Round Loch of Glenhead) and one from a loch with a partially afforested catchment (Loch Grannoch, afforested in 1962-4 and 1977-8) (Fig. 1) are given in Table 1. Both lakes are situated entirely on granite, soils are mainly blanket peats and both lakes have similar modern pH ranges.

Cores ~80 cm long were taken at the deepest point in each lake. The sediment was extruded at 0.5 cm or 1 cm intervals. ²¹⁰Pb measurement follows Häsänen²⁵ and dates were calculated according to the constant rate of supply model^{26,27}. The species frequency composition of the diatom assemblages was analysed using standard techniques²⁸. Over 140 taxa were identified at the two sites. A selection of those that show significant stratigraphical changes through the core sequence at each site are shown in Fig. 2 together with ²¹⁰Pb dates and pH curves calculated to an accuracy of ~±0.3 units using Index B of Renberg²⁴.

For Round Loch of Glenhead the ²¹⁰Pb chronology indicates that the rate of sediment accumulation at this site is 1 mm yr⁻¹ and has been approximately constant over the past 200 yr with unsupported ²¹⁰Pb concentrations reaching background levels at ~25 cm. If this mean rate were extrapolated the 80 cm core covers approximately the past 700 yr of accumulation. It can be seen for the period spanning approximately AD 1300 to 1600 (80-40 cm) that the diatom flora of the lake was dominated by *Anomoeoneis vitrea* (Grun.) Ross, *Eunotia veneris* (Kütz.), O. Mull., *Fragilaria virescens* Ralfs, *Cyclotella kützingiana* Thwaites and *Melosira distans* (Ehr.) Kütz. There was little significant change within the period and the pH reconstruction gives a value of ~5.7. Some time before about AD 1600 (40 cm) there was a strong change in the composition of the flora as *C. kützingiana* and *C. arentii* Kolbe declined rapidly. The reason for this change is not clear but *C. kützingiana* is a planktonic diatom found mainly in water with circumneutral pH. Planktonic diatoms seem to be rare in waters below ~5.5 (D. Charles, personal communication) and the reconstructed pH curve suggests that there was a slight increase in acidity at this time. As the change predates the beginning of significant fossil fuel combustion it is likely to be related either to a climatic, or more probably, to a land-use change.

Between ~1600 and ~1850 (40-15 cm), fairly stable conditions are indicated but a second floristic change begins from about 1850 (15 cm). The following changes are sequential and indicate a progressive decline in pH from the mid-nineteenth century to the present. The first species to change were *Fragilaria virescens* and *Achnanthes microcephala* (Kütz.) Grun., both of which declined. *Anomoeoneis vitrea* also declined, but more gradually and there were reductions in *Nitzschia perminuta* Grun. (at 13 cm), *Navicula coconeiformis* Greg (at 10 cm), and *Cymbella gracilis* (Rabh.) Cleve (at 5 cm), all taxa associated with less acid waters. These taxa were replaced by the increase of species typical of more acidic waters especially *Tabellaria quadrisepitata* Knudsen, *T. binalis* (Ehr.) Grun., *Eunotia veneris*, *E. alpina* (Naeg.) Hust, *E. arcus* Ehr., *E. bactriana* Ehr., *E. tenella* (Grun.) Hust, and *Navicula höfferi* Cholnoky. The reconstructed pH curve shows a continuous but variable rate of decline in pH from about 1850 to the present day from about 5.5 to about 4.6. The data indicate that the rate has been more rapid since 1900 (about 0.01 pH unit yr⁻¹). The calculated pH value of 4.8±0.3 from the surface sediment sample at this site agrees well with the present measured pH of the lake water (see Table 1).

Although the cause of the recent acidification at this site is not known, it is possible to eliminate ploughing and afforestation as factors. Our unpublished historical, palynological and lithostratigraphical data contain no evidence for significant alteration in land-use practice that might have promoted acidification in this lake and therefore the most likely cause is an increase in acidity deposited from atmospheric sources, a supposition that is consistent with the post-Industrial Revolution timing of the changes.

The core from Loch Grannoch covers a much shorter time period than that from Round Loch of Glenhead since the sediment accumulation rate is faster. This is especially so for the top 30 cm where the accumulation rate increases to 1 cm yr⁻¹ as a result of accelerated soil erosion associated with pre-afforestation ploughing. Consequently, the whole 80 cm falls within the ²¹⁰Pb dating range and includes sediment accumulated from about 1840 to the present. Some of the floristic changes are similar to those at Round Loch of Glenhead but the recent history of Loch Grannoch is complicated by the additional effects over the past 20 yr of soil erosion and afforestation.

Figure 2b shows that there was little floristic change in Loch Grannoch between 1840 and about 1930. Except for the presence of two additional taxa, *Eunotia pectinalis* v. *minor* (Kütz.) Rabh. and *Achnanthes marginulata* Grun., that probably reflect the greater importance of epipsammic habitats in Loch Grannoch, the flora of this period (Fig. 2b, 80-40 cm) is similar to

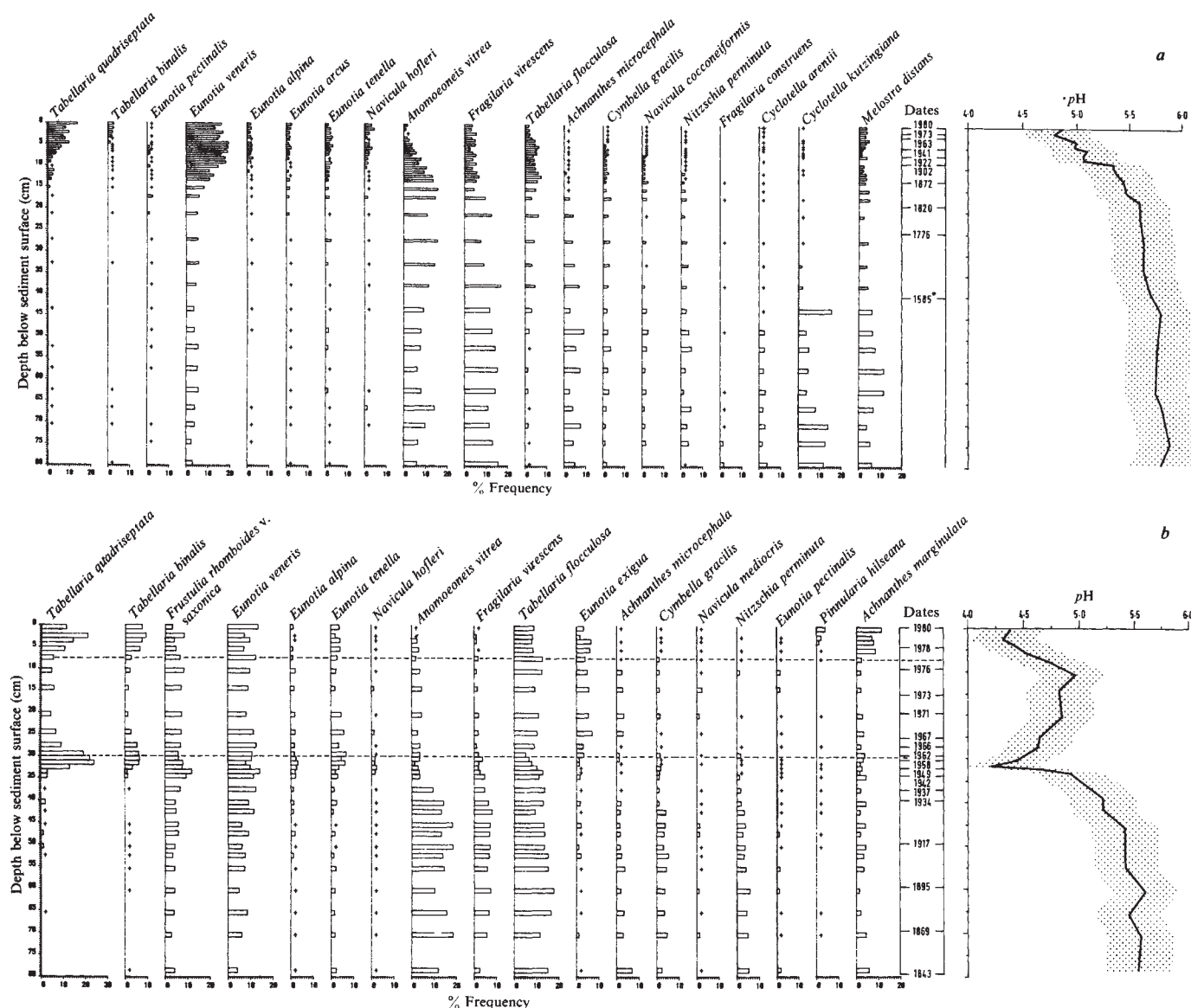


Fig. 2 *a*, Diatom diagram for Round Loch of Glenhead showing percentage frequency of selected taxa, ^{210}Pb dates and pH values using Index B of Renberg and Hellberg¹⁷. Stipple on either side of pH curve indicates ± 0.3 unit boundary. *, Extrapolated date. *b*, Diatom diagram for Loch Grannoch, as for *a* but including dates of pre-afforestation ploughing in 1961 and 1977 indicated by the dashed lines.

that at Round Loch of Glenhead during the previous 300 yr (Fig. 2*a*, 40–20 cm), and represents a lake pH of 5.5 ± 0.3 . The first evidence of acidification occurs within this period at about 1910 (50 cm) and is indicated by a slight shift in the composition of the assemblage. *Nitzschia perminuta* and *Achnanthes microcephala* decline, and *Frustulia rhomboides* v. *saxonica* (Rabh.) de Toni, an acidophilous form, increases. An acceleration in the process occurs in the 1920s and 1930s as *Anomoeoneis vitrea*, *Fragilaria virescens*, *Nitzschia perminuta*

and *Cymbella gracilis* decline, paralleling identical but earlier changes in the Round Loch of Glenhead core (see Fig. 2*a*, 10–15 cm) and indicating a decline in pH to ~ 5.0 by 1940. The most marked change in the flora, however, occurs between 1940 and 1960 (35–30 cm) as the two acidobiontic species *Tabellaria binalis* and *T. quadrisepiata* appear and rapidly expand, representing a further and more rapid decline in pH values to levels similar to those of today. Overall, the floristic changes from 1910 to 1960 suggest that there was a drop of

Table 1 Selected characteristics of the catchments

	Altitude (m)	Loch area (ha)	Maximum depth (m)	Catchment* area (ha)	Catchment: loch*	% Afforested	pH range†	Na ⁺ ‡	Ca ²⁺	Mg ²⁺	Al ³⁺	Cl ⁻	SO ₄ ²⁻	NO ₃ ⁻
Round Loch of Glenhead	299	12.6	13.5	95	8.6:1	0.0	4.5–5.0	113	30	41	13	136	111	6
Loch Grannoch	210	114.3§	21.1	1287	12.3:1	69.5§	4.4–4.9	152	50	50	30	165	152	8

* Excludes loch area.

† Range of eight values from samples taken at intervals from November 1981 to November 1982.

‡ Water chemistry values are taken from ref. 16. Concentrations in $\mu\text{equiv. l}^{-1}$.

§ Includes 510.8 ha (39.7%) afforested in 1962–64, and 376.4 ha (29.8%) afforested in 1977–78.

~1 pH unit from ~5.5 to ~4.5 during this period. As the change predates the beginning of afforestation (1962) in the Loch Grannoch catchment this acidification cannot be ascribed to afforestation processes, and as there is no historical evidence for a significant change in other land-use practices during this period it is most likely, as at Round Loch of Glenhead, that the increased acidity was derived from atmospheric sources.

In 1961 the eastern part of the Loch Grannoch catchment was ploughed before planting in 1962 (Fig. 1). The effect is clearly registered in the sediment by a sudden increase in sediment accumulation derived from a sharp decrease in ^{210}Pb concentration at the 29–30 cm level. It can be seen (Fig. 2b) that immediately after the onset of accelerated erosion there is a strong decrease in *Tabellaria binalis* and *T. quadrisepitata*, followed by a recovery to higher values in the upper 8 cm (from 1977). In the top few centimetres *Anomoeoneis vitrea* declines to trace levels and *Pinnularia hilseana* (Janish) Müll. and *A. marginulata* expand. Index B suggests that these diatom changes reflect a reversal in the trend of pH values from <4.5 in 1960 to ~5.0 in the mid-1970s followed by a second decline to modern values (4.4–5.0, Table 1) over the past 5–6 yr. So far we have no firm explanations for this observation. However, the temporary rise of pH is probably an artefact caused by the addition of a significant quantity of diatoms from inwashed catchment peats depressing the percentages of *Tabellaria binalis* and *T. quadrisepitata*. These are acidobiontic taxa that are not characteristic of catchment diatom floras.

Despite some uncertainty concerning the effects of afforestation several firm conclusions can be made from the results obtained at the two sites. First, the contemporary level of acidity in the lakes studied so far is a result of acidification during the late nineteenth and twentieth centuries. Second, the lakes were already acid (pH < 6.0) before the recent changes took place and a change in water quality involving a slight acidification is apparent at Round Loch of Glenhead about 400 yr ago. Third, the recent acidification has occurred in lakes with unafforested as well as afforested catchments. And fourth, although the diatom changes were similar at both sites, the timing and rates of acidification were different, suggesting that the acidification process is at least in part controlled by catchment characteristics.

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Circadian clock in *Xenopus* eye controlling retinal serotonin *N*-acetyltransferase

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Circadian rhythms are controlled by endogenous oscillators or clocks. These clocks exhibit a persistent period of approximately 24 h in constant conditions, a specific phase relationship to a periodic cue (zeitgeber) in the external environment, and plasticity in that the phase of the clock may be altered in response to a phase change in the zeitgeber¹. Although many processes exhibit circadian rhythmicity, the nature and location of endogenous clocks remain poorly defined. Recent evidence in vertebrates suggests that the mammalian suprachiasmatic nucleus and the avian pineal gland contain clocks that affect the rhythmicity of indoleamine metabolism². The vertebrate retina also exhibits a circadian rhythm of serotonin *N*-acetyltransferase activity (NAT, EC 2.1.1.4)^{3–5}, a key enzyme controlling melatonin synthesis, and of photoreceptor disk shedding^{6,7}. The latter process may be regulated by melatonin⁸, and the immediate cellular events seem to be controlled locally within the eye^{9–11}. Although sustained oscillation and entrainment were not demonstrated, data suggesting that an ocular circadian clock influences disk shedding have been reported⁹. We sought evidence for an ocular clock by studying retinal NAT activity in *Xenopus* eye cups maintained in culture and report here both sustained oscillation and entrainment of the *in vitro* system. The data indicate that in addition to the suprachiasmatic nucleus and pineal gland, the eye itself must be regarded as the locus of a circadian clock in vertebrates.

Postmetamorphic, juvenile *Xenopus laevis* (Nasco, Fort Atkinson, Wisconsin) were maintained in our laboratory for a minimum of 1 month on a 12-h light, 12-h dark (LD 12:12) schedule at 23 ± 1 °C before use in experiments. We have previously shown that the retina of this species exhibits a robust rhythm of NAT activity which peaks during the dark phase of the light cycle, persists for at least 3 days in constant darkness and is blocked in constant light⁵. We have also found that retinal NAT activity shows a dark-dependent increase during the subjective dark period in eye cups cultured⁵ in conditions previously used to examine the physiology of photoreceptor membrane turnover¹¹. We therefore used this culture system to determine whether a circadian clock exists in the eye.

Eye cups were prepared by surgical removal of the cornea, iris and lens in room light during the 1-h period ending 2 h before light offset, and were transferred to amphibian tissue culture medium supplemented with sodium bicarbonate (30 mM)¹¹ and ascorbic acid (100 µM). Eye cups were placed in 4 ml of medium in 35-mm Falcon culture dishes (five per dish) inside a plastic culture chamber which was sealed with a moist atmosphere of 95% O₂:5% CO₂. The medium pH at equilibrium was 7.4. The chamber was rotated at 60 r.p.m. on an orbital shaker inside an incubator at 21 ± 0.5 °C. At the time of light offset retinas were removed from eye cups and immediately frozen on dry ice. In each of our experiments samples (*n* = 5 per time point) were taken at 6-h intervals for 30 h, beginning at the time of normal light offset in the laboratory. Frozen retinas were subsequently homogenized and NAT activity was assayed by measuring the transfer of a [¹⁴C]-acetyl group from acetyl-coenzyme A to tryptamine⁵.

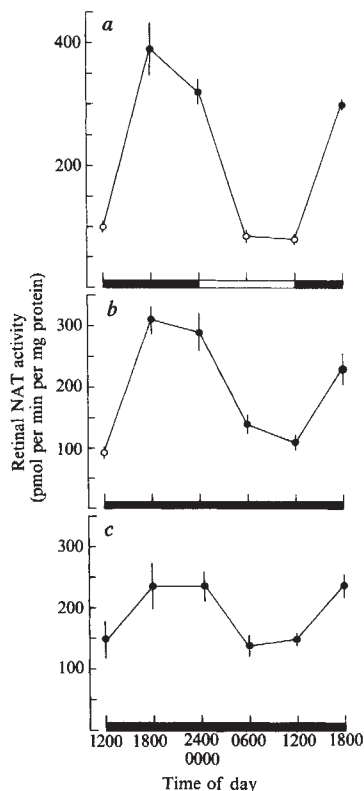


Fig. 1 Data demonstrating sustained oscillation of retinal NAT activity in eye cups maintained in culture. Light cycle in *a* was controlled with a timing device. Light from two 25-W incandescent bulbs had an intensity of $1.8 \times 10^{-3} \text{ W cm}^{-2}$ incident at the level of culture dishes. Due to a 'greenhouse effect', the temperature inside the culture chamber rose gradually during the light period to 24 °C. It was constant at 21.5 °C during the dark periods in *a* and throughout the experiments plotted as *b* and *c*. Points represent means ($n = 5$) and vertical bars correspond to 1 s.e.m. on each side of the mean. Open circles represent retinas prepared in light, and filled circles represent those prepared in dim red light (Kodak Wratten No. 2 filters). The first time point (1200 h) is the time of normal light offset. In *a* and *b* this corresponds to a period 2 h after preparation of cultures whereas in *c* it corresponds to 50 h after culture preparation. Each time a sample was removed from the culture chamber the culture medium of all remaining samples was changed. During the 2 days preceding the first sample in *c* the medium was changed once daily at 0600 h. NAT activity was assayed as described previously⁹ except that the reaction product was extracted into 1 ml of toluene/isoamyl alcohol (97:3). All data were analysed statistically using analysis of variance and the rhythms in each case were significant (for *a* and *b*, $P < 0.001$; for *c*, $P < 0.01$).

When the eye cups were kept on a normal light-dark schedule *in vitro*, retinal NAT activity exhibited a nearly fourfold rise during the first dark period, a decline back to baseline levels during the light period and a subsequent threefold rise at the beginning of the second dark period (Fig. 1*a*).

When the experiment was repeated using eye cups maintained in darkness throughout the 30-h culture period, a similar rhythm was detected with peak activity in subjective darkness and near baseline activity in subjective light (Fig. 1*b*). Although damped in amplitude, the rhythmic activity of NAT persisted during the third day of culture in constant darkness as well (Fig. 1*c*). The damped rhythm at 3 days showed both increased baseline NAT activity in subjective light and decreased peak activity in subjective darkness compared with day 1 in darkness (compare Fig. 1*b* and *c*). These data demonstrate sustained oscillation of NAT activity *in vitro* and suggest that a clock controlling that activity exists in the eye.

To determine whether the phase of the *in vitro* rhythm of retinal NAT activity could be altered by a change in light cycle,

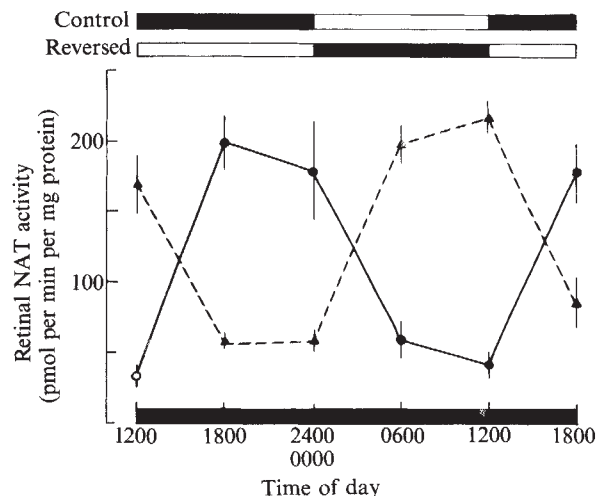


Fig. 2 Data showing phase reversal of the NAT rhythm *in vitro*. Groups of eye cups were kept through two complete 24-h cycles *in vitro* on either a control cycle identical to that to which animals were entrained (solid line, circles) or to a reversed cycle (broken line, triangles). On the first day in culture dark onset was delayed 12 h. Light was delivered as in Fig. 1*a*. Samples were taken at 6-h intervals over a 30-h period in constant darkness (shaded on abscissa) beginning 50 h after cultures were prepared. The bars at the top indicate the periods of subjective light and dark for control and reversed groups. Data are plotted as in Fig. 1. The rhythms were significant ($P < 0.001$) as determined by analysis of variance.

we carried out phase reversal experiments *in vitro*. Eye cups were prepared as above and maintained for 48 h under a lighting regime (LD 12:12) identical to that of the animals from which they were obtained or in a lighting regime with light and dark periods reversed (Fig. 2). After two complete 24-h cycles *in vitro*, such eye cups were maintained in constant darkness beginning at the time of subjective light offset in the normal cycle, and samples were taken for assay at 6-h intervals. Eye cups maintained on a light cycle identical to that used for entrainment of the animals exhibited a rhythm in constant darkness with peak NAT activity during subjective darkness of the entrained cycle (Fig. 2). In contrast, eye cups kept on a reversed cycle *in vitro* exhibited peak NAT activity during periods corresponding to the dark period of the immediately preceding *in vitro* light-dark cycle (Fig. 2). This observation suggests that the retina contains a circadian clock whose phase can be altered in response to a change in phase of a zeitgeber. The rapid phase reversal is similar to that reported for chick pineal gland¹².

The above experiments were controlled for possible extraneous cues (culture medium changes or temperature fluctuations) that could influence maintenance (Fig. 1) or phase-shifting (Fig. 2) of the *in vitro* rhythm. First, culture medium for all samples was changed at each sample time (6-h intervals). Thus, except for the first samples (1200 h) in Fig. 1*a* and *b*, each sample received a culture medium change 6 h before freezing. Second, in the phase shift experiments culture medium was changed once daily during the 2-day phase shift period but for control and reversed groups this change occurred at the same time of day (0600 h) relative to the control (pre-experimental cycle). To control for culture medium changes on days 1 and 2 of culture acting as a cue for maintenance of the dark rhythm at 3 days of culture (Fig. 1*c*), experiments were carried out with such medium changes occurring at the middle of the subjective-dark (Fig. 1*c*) or subjective-light period (data not shown). In both cases the phase of the dark rhythm was determined by the phase of the entraining light-dark cycle and not by the phase of daily changes of culture medium. Third, due to a slight 'greenhouse effect' in our initial phase shift experiment (Fig. 2) which raised the temperature 2.5 °C during the light period, we repeated the experiment using fluorescent

lighting ($8.5 \times 10^{-4} \text{ W cm}^{-2}$ incident at culture dishes), a continuous flow of cooled gas (0.5 l min^{-1} of 95% O_2 :5% CO_2) through the culture chamber and continuous temperature monitoring. During the phase shift period (48 h), temperature was maintained at 22°C and fluctuated by $<0.5^\circ\text{C}$. Despite the lower light intensity used, complete phase reversal was observed. Experimental samples at each time point were not significantly different ($P > 0.05$) from those illustrated for the reversed group in Fig. 2. Therefore, the experimental data suggest that an endogenous clock in eye cups can be phase-reversed by light received through local photoreceptors.

The NAT activity rhythm in *X. laevis* presumably controls the rhythmic synthesis of melatonin, as occurs in the pineal gland¹³ and chicken retina⁴. Although molecular mechanisms remain undefined, melatonin may be an effector of avian and mammalian behavioural rhythms^{14,15}. It is also involved in the photoperiodic control of reproduction in mammals¹⁶. A general role for ocular melatonin remains to be determined. One possibility is that it is involved in the local control of other aspects of rhythmic activity of the eye. Retinomotor movements of pigment epithelium and photoreceptors¹⁷, photosensitive membrane turnover^{6,7} and b-wave amplitude of the electroretinogram¹⁸ all exhibit free-running circadian rhythms in constant darkness. Although reports are inconclusive, retinomotor movements have been reported to be influenced by melatonin (reviewed in ref. 19). In addition, we have recently demonstrated that photoreceptor disk shedding can be specifically activated by melatonin⁸. These observations suggest that the ocular NAT rhythm functions in rhythmic melatonin metabolism, which in turn affects rhythmic aspects of photoreceptor metabolism. For photoreceptor disk shedding this view is further supported by data suggesting that a local timing mechanism influences disk shedding in eyes of *X. laevis* in culture²⁰.

In rodent pineal glands, rhythmic activity of NAT requires input from the suprachiasmatic nucleus via its sympathetic innervation². The suprachiasmatic nucleus seems to be coupled to the external day-night cycle through retinal photoreceptors² and itself exhibits properties of a circadian clock²¹. In chickens, however, rhythmicity of pineal NAT activity persists in constant conditions in culture^{12,22-24}, and alterations in the light-dark cycle results in a phase shift of the rhythm¹². This suggests that both photoreceptors and circadian clock are located within the avian gland itself.

The data presented here provide direct evidence for a circadian clock in the vertebrate eye, and in this regard suggest a degree of similarity to the avian pineal gland¹² and *Aplysia* eye²⁵. The extent to which such clocks operate autonomously in the intact animal remains to be determined. On the basis of eye patch and optic nerve lesion experiments, it has been suggested that each eye of the rat contains a circadian clock which controls photoreceptor disk shedding⁹. However, phase shifting of the ocular rhythm appeared to require an intact optic nerve and presumably central processing of the phase shift signal. Our results differ in that they demonstrate both sustained oscillation and phase reversal *in vitro*. It will be of future interest to determine the extent to which the same oscillator controls both disk shedding and NAT activity as well as the extent of interaction of the ocular clock with other oscillators of the vertebrate circadian system.

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Regulation of axon number in primate optic nerve by prenatal binocular competition

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We report here that in mature rhesus monkeys in which one eye was removed during the first half of gestation, the optic nerve of the remaining eye is larger and contains significantly more retinal axons than in age-matched control animals. Such supernumerary fibres in monocularly enucleated monkeys probably result from an arrest in the normal process of elimination of excess embryonic optic axons¹. Although the function of retained supernumerary optic axons is unknown, this finding demonstrates that (1) competition between the two eyes for synaptic territory occurs prenatally, before visual experience and (2) an early lesion in one brain area can adjust or enhance the size and perhaps the performance of other synaptically related structures.

Each eye in the adult human gives rise to about 1 million optic fibres that are distributed approximately equally to the two sides of the brain² and terminate in the dorsal lateral geniculate nucleus (LGNd) in the six separate layers³. Three laminae (1, 4 and 6) receive input from the contralateral eye; the remaining three (2, 3 and 5) receive input from the ipsilateral eye. As a similar neuronal organization exists in the rhesus monkey^{4,5}, it is possible to study development of the binocular visual system experimentally. Thus, in fetal monkeys projections from the two eyes to the LGNd have been shown to initially overlap before segregating into separate layers during the third quarter of gestation^{6,7}. During the period of overlap, each optic nerve contains about 2.85 million axons, more than twice the number present in the adult¹. Elimination of the excess 1 million optic axons also occurs within the third quarter of gestation and therefore is synchronized with the segregation of retinal inputs to the LGNd.

The temporal coincidence of axon elimination and segregation of retino-geniculate terminals suggests that axons from one eye may be selectively eliminated from layers 2, 3 and 5 in the ipsilateral and from layers 1, 4 and 6 in the contralateral LGNd¹. The segregated arrangement of axon terminals in the normal adult LGNd may be achieved by competition between axons from the left and right eyes for appropriate synaptic space within the developing LGNd. The selective elimination hypothesis was proposed on the basis of the finding that when one eye is removed during the first half of gestation the remaining eye projects indiscriminantly to the entire LGNd in the adult⁸. However, this diffuse pattern could be achieved by expansion of retinal terminals from appropriate to inappropriate territories rather than by the retention of supernumerary embryonic axons due to the absence of competition from the

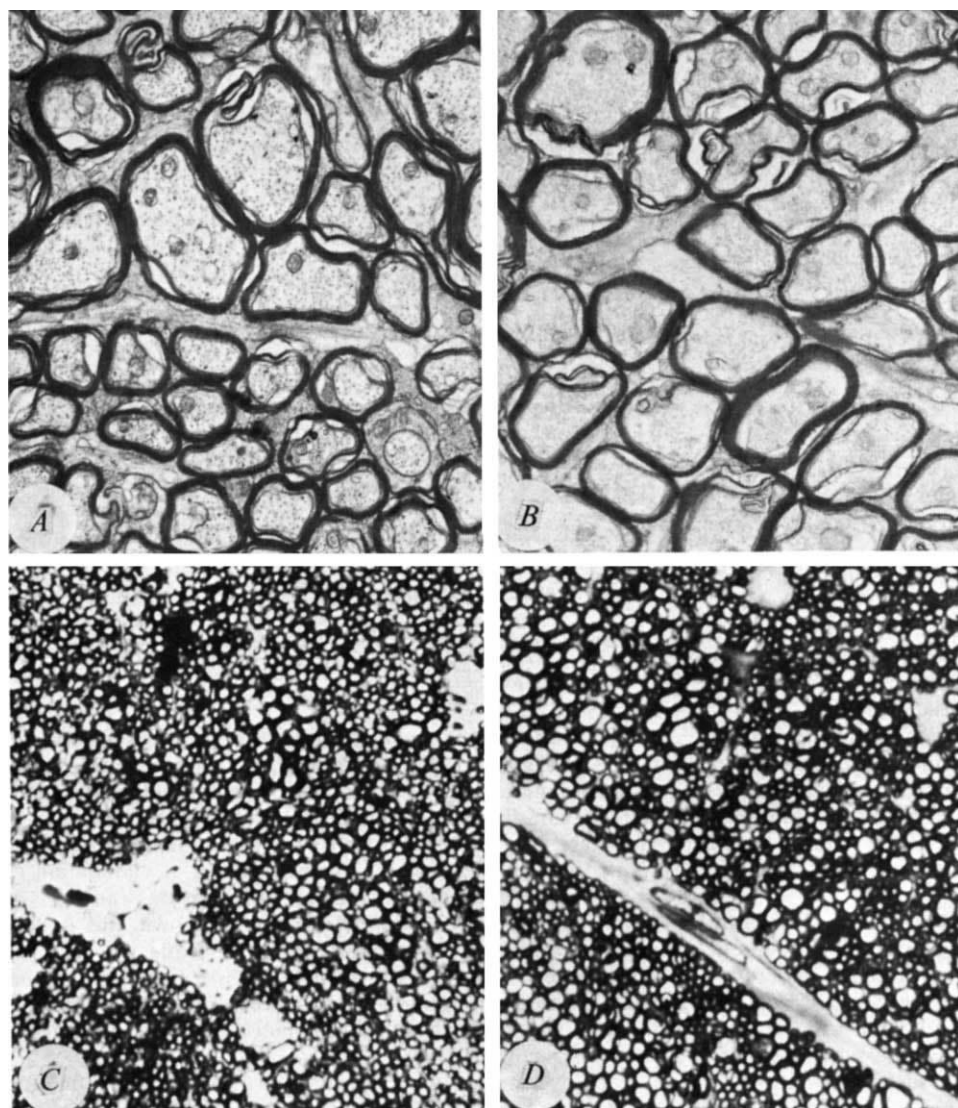


Fig. 1 A, Electron micrograph of the optic nerve in a 68-day-old monkey (P68) that was enucleated on the 63rd embryonic day (E63). B, Electron micrograph of the optic nerve in the control (binocular) animal at P67. A, B, $\times 12,600$. C, Light microscopic photograph of the optic nerves in P361 monkey that was enucleated at E65; D, similar photograph in control animal (P335). C, D, $\times 2,450$.

Methods: In the enucleated and control monkeys killed at P68 and P67 respectively, adjacent thin (500 Å) sections were placed on Formvar-coated slot grids and stained with uranyl acetate and lead citrate for electron microscopic analysis. Photomontages from a series of consecutive overlapping electron micrographs (A, B) were made across two diagonal diameters of the optic nerve at a final magnification of $\times 20,000$. In the enucleated and control animals that were killed at P341 and P335 respectively, optic axons were counted from light microscopic photomontages across two diagonal diameters of optic nerve. Photographs were taken using a Zeiss $\times 100$ oil immersion objective (C, D). As by 1 yr old, all retinofugal axons in the monkey are myelinated (ref. 9 and unpublished observation by P.R.) counts of optic axons at the light microscopic level in osmium-treated preparations are accurate and reliable at this age. For all animals, the number of axons per unit area was determined from the photomontages and their total number estimated as described previously¹.

contralateral eye. The possibility of retention of supernumerary axons can be tested directly by counting fibres in the optic nerve of the intact eye of an animal subjected to monocular enucleation during embryonic life. If the number exceeds that present in the optic nerve of normal animals it would strongly support the selective elimination hypothesis.

The optic nerves from two prenatally enucleated and two control monkeys of corresponding ages were analysed (Table 1). Prenatal enucleation was performed in the first third of pregnancy (embryonic (E) days E63 and E65). Pregnant females were subjected to a hysterotomy, the fetal heads were exposed, and the left eye was dissected out. The head was then returned to the womb and fetal membranes, uterine and abdominal walls were sutured as described previously^{6,7}. Pregnancies were carried to full term (E165), then the fetuses were delivered by caesarean section and allowed to survive for either 2 (P68) or 11 months (P341). Age-matched unoperated monkeys were

killed at P67 and P335. Following vascular perfusion with 1% paraformaldehyde and 1.25% glutaraldehyde in phosphate buffer, the optic nerve was dissected out midway between the eyeball and the chiasm, postfixed in OsO_4 and embedded in Epon-Araldite. Thick (1–2 μm) transverse sections stained with toluidine blue were prepared to determine the cross-sectional area of each nerve using a Zeiss-MOP-3 digitized tablet.

The cross-sectional area of the optic nerve in the 2-month-old enucleated monkey was 3.75 mm^2 or $\sim 15\%$ greater than that of the optic nerve of the age-matched control animal (Table 1). However, axonal counts revealed that 2.33×10^6 axons were present in the remaining optic nerve of the enucleated monkey compared with 1.69×10^6 in the age-matched control. Thus, at 2 months old, the optic nerve of an enucleated monkey contains 37.8% more axons than a normal monkey of comparable age¹. The proportionally larger number of fibres in the optic nerve having a slightly small cross-sectional area is probably due to

Table 1 Optic nerve area and axon number in prenatally enucleated and control monkeys

Case	Day of enucleation	Day of killing	Optic nerve area (mm^2)	Difference in area	No. of axons ($\times 10^3$)	Difference in number
a	E63	P68	3.75	14.6%	2,330	37.8%
b	—	P67	3.27		1,690	
c	E65	P341	6.27	32.2%	1,810	31.6%
d	—	P335	4.74		1,380	

Two monkeys (a and c) were subject to monocular enucleation at embryonic (E) days E63 and E65 respectively, and killed at postnatal (P) days P68 and P341. Unoperated control monkeys (b and d) were killed at P67 and P335.

the presence of more smaller diameter axons in the enucleated animal than in the control. In the enucleated monkey, the small axons run in bundles (Fig. 1A)—one possibility is that these smaller axons represent the vestige of the embryonic projections that transiently innervate inappropriate laminae. They survive in the enucleated monkey and their smaller diameter may be due to the smaller size of terminals and/or to fewer synapses with inappropriate LGNd neurones.

Similar results were obtained for the 11-month-old enucleated monkey (Table 1), in which the cross-sectional area of the optic nerve was 6.27 mm², 32.2% larger than that of the age-matched control animal (4.74 mm²). The percent difference in cross-sectional area was twice that observed between experimental and control animals killed at P68. The total number of axons in the monocular animal was 1.81×10^6 (31.6% more than in the control animal). This difference is only slightly changed from that observed at younger ages but total counts indicate a continuous elimination of axons in both the enucleate and control animal (Table 1).

The 37.8% and 31.6% increases in optic fibres found in enucleated monkeys far exceed the maximum variation attributable to individual differences (13%) recorded by any single method of counting in normal rhesus monkeys^{1,9-11}, thus the increase is statistically significant. Our findings are consistent with the available literature: monocular enucleation in newborn rodents reduces the naturally occurring degeneration of retinal ganglion cells^{12,13}, and prenatally enucleated kittens have larger than normal numbers of optic axons¹⁴. During normal development the number of optic axons in the monkey exceeds 2.8×10^6 ; therefore, supernumerary axons in prenatally enucleated monkeys probably result from reduced axonal elimination in fetal life rather than from overproduction. However, the finding that less than half of the supernumerary axons that were present in the single fetal optic nerve at the time of enucleation¹ survive to postnatal ages indicates that multiple factors may control their final numbers. For example, although initially supernumerary axons mainly innervate inappropriate LGNd laminae, at later developmental stages some contribution of retinal terminals may spread from the appropriate layers to adjacent laminae and become engaged in axonal competition¹⁵. Elimination of optic axons occurs mainly in the third quarter of gestation, but continues at a low level postnatally as more fibres were present in the 2-month-old animal than in the 11-month-old animal although both enucleations were performed at the same time in gestation when optic axons are most numerous.

Apart from advancing our understanding of the genesis of visual connections in humans, the present findings illustrate a general principle, namely that one population of neurones can control the number of neurones in another structure with which it shares a common synaptic territory. It is tempting to speculate that similar competitive mechanisms may underlie compensatory enlargement or possibly even the enhancement of function of one brain structure when another structure is damaged during development. More detailed knowledge about timing and regulation of this synaptic interdependence is essential for understanding normal neural development, pathogenesis of cerebral malformations and interpretation of perinatal brain injuries.

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Dopamine visualized in the basal ganglia of living man

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The neurotransmitter dopamine has biological attributes that make it amenable to study by positron emission tomography, unlike many of the 40 or so neurotransmitters that have been identified in the brain. Dopamine deficiency in the nigrostriatal system is a characteristic of Parkinson's disease¹, and a disturbance of dopamine metabolism is still widely held to be responsible for the syndrome of schizophrenia². Despite its importance in the regulation of locomotion and mood, it has been impossible to visualize the intracerebral distribution of dopamine and measure its regional metabolism in man. In the first demonstration of the regional distribution of a neurotransmitter in the brain of conscious normal man, we show here that L-3,4-dihydroxyphenylalanine (L-dopa) labelled in the 6-position with the positron-emitting radionuclide fluorine-18, localizes specifically in the dopaminergic pathways of the human brain where its turnover could be measured atraumatically by positron emission tomography.

The cell bodies of the central dopaminergic neurones of mammals are located in the substantia nigra and ventral tegmental area³. They project to the striatum as well as to the frontal, cingulate and olfactory cortices⁴. These neurones synthesize dopamine *in situ*⁵. Dopamine cannot cross the blood-brain barrier and must be synthesized by decarboxylation of its immediate precursor, L-dopa. This amino acid does cross the barrier and is used to treat Parkinson's disease⁶. It is normally synthesized *in situ* from tyrosine. Dopa, the immediate dopamine precursor, has been labelled with the positron-emitting radionuclide fluorine-18 (refs 7, 8). ¹⁸F-5-fluoro-DL-dopa behaves as an analogue of natural dopa⁹ and, when given intravenously, crosses the blood-brain barrier with kinetics similar to those of dopa¹⁰. However, the *O*-methylation of this tracer is accelerated because the F atom is close to the hydroxyl groups of fluorodopa¹¹. Consequently, we selected ¹⁸F-6-fluoro-L-dopa as a tracer for measurements of dopa distribution in man.

A positron emission tomograph comprising 160 bismuth germanate crystals¹² was used to study three normal male members of the laboratory staff. Their ages were 28, 49 and 50 yr. None was taking any medication. Each study was done in a quiet dimly lit room but no special effort was made to control sensory input. Subjects lay in the tomograph with their head aligned so that the basal ganglia were always included in the plane of view. 100 MBq (specific activity 6 GBq mmol⁻¹) ¹⁸F-6-fluoro-L-dopa was injected intravenously after the subject had been positioned in the tomograph, and sequential images of the distribution of ¹⁸F were obtained over the next 3 h. Figure 1 is a typical picture obtained 3 h after the injection of ¹⁸F-6-fluoro-L-dopa and shows that the major accumulation of ¹⁸F is in the striatum. The radionuclide is also concentrated in the anterior cingulate and frontal cortex.

The cerebral capillaries of mammals contain varying amounts of aromatic amino acid decarboxylase¹³. Consequently, two of the subjects were restudied after taking the peripheral decarboxylase inhibitor L-methyl dopa hydrazine (carbidopa). 100 mg carbidopa were taken orally 90 min before ¹⁸F-fluoro-dopa, 50 mg were taken 30 min before and another 50 mg were

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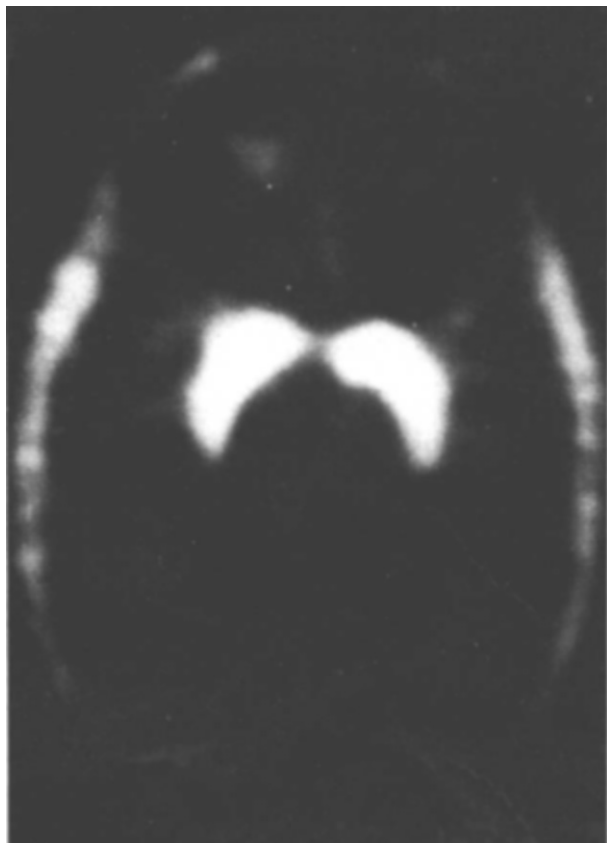


Fig. 1 Tomographic section, face uppermost, obtained in man 3 h after the intravenous injection of ^{18}F -6-fluoro-L-dopa. The section was taken parallel to and 3 cm above the orbito-meatal line. It shows specific accumulations of ^{18}F (white) in the striatum and to a lesser degree in the anterior cingulate and frontal cortex. The accumulation of ^{18}F in the perimeter is due to scalp.

taken immediately before the radiopharmaceutical was injected. These doses of carbidopa were derived from its pharmacokinetics¹⁴ and would have completely blocked the decarboxylase activity of the endothelium of the cerebral microvasculature. Nevertheless, the distribution of ^{18}F in the tomographic slice that contained the striatum remained unchanged. The localization of ^{18}F in the striatum and cortex was therefore not merely a reflection of aromatic acid decarboxylase activity in the capillaries of grey matter structures.

As in any external measurement of the accumulation of a radioactively labelled tracer, information is only obtained about the label itself. In the present study we believe that the distribution of ^{18}F in the tomographic slices truly reflects the distribution of the dopaminergic pathways. The strength of the carbon-fluorine bond prevents ^{18}F from leaving its position on the benzene ring¹⁵. ^{18}F -fluorodopa is transported into the brain¹⁰ and is a substrate for cerebral aromatic acid decarboxylase⁹. Its striatal decarboxylation and vesicular storage as ^{18}F -fluorodopamine can be inferred from observations in monkeys that reserpine discharges striatal ^{18}F which has accumulated after the injection of ^{18}F -6-fluoro-L-dopa¹⁶. Reserpine releases catecholamines from their presynaptic storage vesicles¹⁷.

In spite of the vast literature that has accrued since Parkinson's disease was shown to be associated with a deficiency of striatal dopamine, we still do not know the function of the striatum. It probably receives processed sensory information and other afferent information from all areas of the cerebral cortex and midline thalamic nuclei and has been viewed as the 'receiving area' of the basal ganglia¹⁸. Dopamine fulfills the criteria of a neurotransmitter¹⁹ but it may also alter the threshold of neurones in dopamine-rich areas of the brain and thus behave as a neuromodulator⁶. Now that striatal dopamine

has been visualized in living human brain, its regional intracerebral metabolism can be measured. Its role in Parkinson's disease, Huntington's disease and some of the syndromes characterized by rigidity, akinesia and dyskinesia must be re-evaluated. The mechanism of the devastating fluctuations in the response to L-dopa therapy that parkinsonian patients suffer is also open to scrutiny with radiofluorine-labelled dopa, as is the notion that schizophrenia may be associated with an irregularity of central dopamine metabolism.

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The mechanism of kainic acid neurotoxicity

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The putative excitatory transmitters glutamate and aspartate, as well as their excitatory analogues, can kill neurones in the central nervous system and may thus be involved in the pathogenesis of various neurodegenerative disorders¹. Several studies have suggested that postsynaptic receptors are important in the mechanism of toxicity². However, presynaptic factors might also be involved because, in some brain areas, the neurotoxicity of kainate (a potent structural analogue of glutamate) is greatly reduced following elimination of afferent excitatory innervation³⁻⁶, even though the postsynaptic excitatory potency of kainate may be unaltered in these conditions⁷. The supply of glutamate from the afferent nerve endings has been suggested to be a necessary factor^{3,6,8}. Recently, Ferkany, Zaczec and Coyle⁹ concluded from studies on slices of mouse cerebellum that kainate activates presynaptic kainate receptors on parallel fibre terminals to release glutamate and that it is the postsynaptic interaction between kainate and the released amino acid that is instrumental in causing neuronal necrosis. The more direct evidence we report here does not support these conclusions.

The main results that led Ferkany *et al.*⁹ to this proposal can be summarized as follows: (1) kainate caused a Ca^{2+} -dependent release of endogenous glutamate, an effect that was reduced in cerebellar slices deficient in granule cells (from which the parallel fibres originate), and (2) D- α -amino adipate, which can inhibit the excitatory action of the parallel fibre transmitter, enhanced the kainate-induced accumulation of cyclic GMP. They interpreted this as suggesting that the antagonist, by blocking the

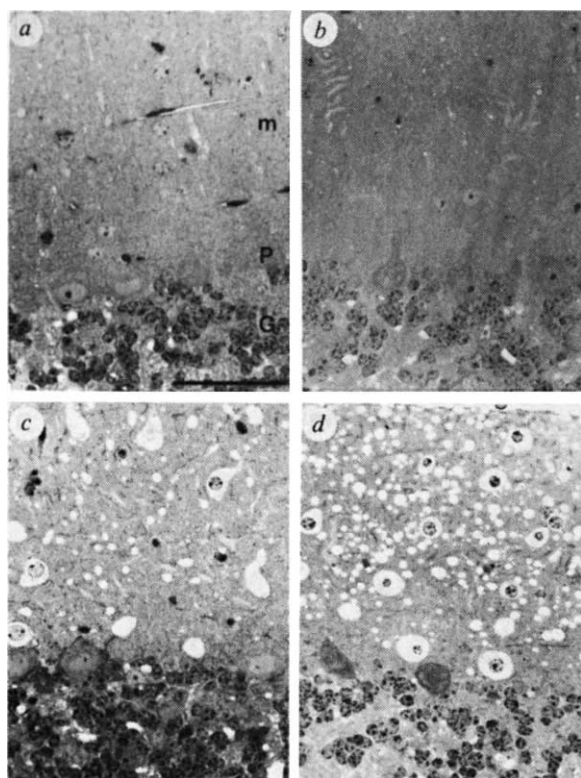


Fig. 1 Light micrographs of adult mouse cerebellar slices incubated in a Krebs-Henseleit medium¹¹ in the absence (a) or presence of kainate at concentrations of 3 μ M (b), 5 μ M (c) and 10 μ M (d) for 1 h. Note that in slices exposed to 5 and 10 μ M kainate, the stellate and basket interneurons in the molecular layer (m) are acutely necrotic and Purkinje cells (P) exposed to 10 μ M kainate have become darker. Granule cells (G) remain intact throughout. Scale bar, 50 μ M.

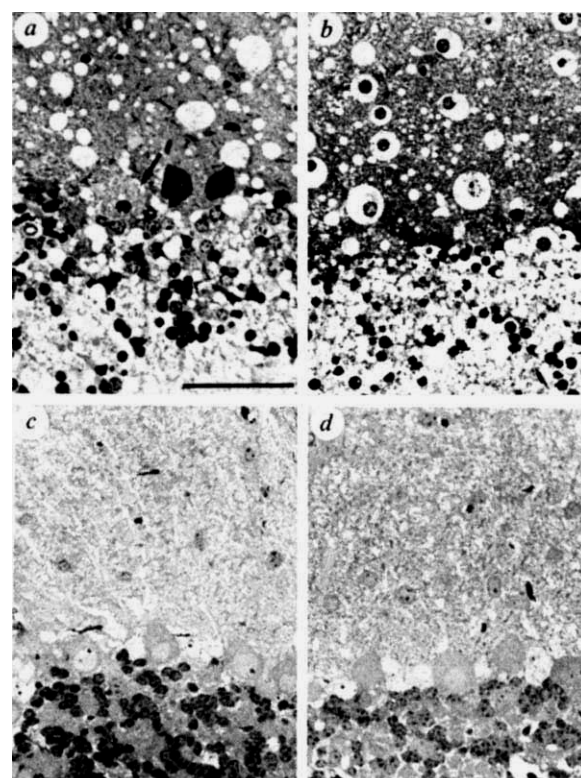


Fig. 2 Mouse cerebellar slices exposed for 1 h to 30 μ M kainate (a), 500 μ M kainate plus 250 μ M D- α -amino adipate (b), 3 mM L-glutamate (c) and 3 mM L-glutamate plus 3 μ M kainate (d). In b, D- α -amino adipate was added 5 min before kainate. The numerous swellings in the granule cell layer in a and b represent expansion of the granule cell dendritic digits¹¹. At 30 μ M kainate (a), in addition to changes in the granule cell layer, several Purkinje cells had become highly vacuolated (arrow), an effect seen more generally at higher concentrations (b). Scale bar, 50 μ M.

postsynaptic action of the released amino acid, prevented interactions between it and kainate that would otherwise have resulted in neurotoxicity (and a truncation of the cyclic GMP response).

In the present experiments, we have sought direct answers to the following questions: are the concentrations of kainate which elicit release of endogenous excitant appropriate to the concentrations causing neurotoxic effects; does D- α -amino adipate inhibit kainate neurotoxicity; and is the availability of glutamate a limiting factor for the toxicity of kainate?

The experiments were carried out on incubated slices of adult mouse cerebellum (as used by Ferkany *et al.*) prepared and incubated as previously described for slices of rat cerebellum¹⁰. After a preincubation period of 1½–2 h, kainate or other agents were added and the incubation was continued for a further hour. The slices were then fixed, dehydrated, embedded in resin and sectioned at 0.5 μ m for light microscopy¹¹.

All the neuronal and glial cell types in the slices incubated in control conditions (Fig. 1a) showed good preservation in areas away from the cut edges (the damage here was about 60 μ m thick). At a concentration of 3 μ M, kainate produced no clear effects on any of the cell types (Fig. 1b). At 5 μ M, however, numerous vacuoles appeared in the molecular layer and inhibitory interneurons (basket and stellate cells) became highly swollen and necrotic (Fig. 1c); 10 μ M kainate resulted in more extensive swelling in the molecular layer and also caused Purkinje cells to appear much darker (Fig. 1d). Throughout these concentrations, granule cells remained intact and well preserved. At 30 μ M, however, the granule cell layer became perforated with numerous swellings and many of the granule cell somata appeared condensed and dark (Fig. 2a).

These results are virtually identical to those described previously for the rat cerebellum¹¹ and show that Purkinje cells

and inhibitory interneurons (the cells innervated by parallel fibres) are more prone to kainate toxicity than are granule cells and that the concentrations of kainate required to elicit neurotoxic effects selectively against the former are 5–10 μ M.

Ferkany *et al.*⁹ suggested from their biochemical data that 250 μ M D- α -amino adipate inhibited the neurotoxicity of 500 μ M kainate. In the present study, the neurotoxic effects of this concentration of kainate were found to be similar to those produced by exposing slices to 30 μ M kainate (Fig. 2a) for a longer time¹¹, that is, necrosis of granule cells was more complete and Purkinje cells had become highly vacuolated. These effects were completely unaffected by 250 μ M D- α -amino adipate (Fig. 2b).

To test whether the availability of glutamate might be important for kainate toxicity, slices were incubated with this amino acid in the absence or presence of a concentration of kainate (3 μ M) just below the minimum amount required to induce neurotoxicity on its own (5 μ M).

The most prominent histological changes observed in slices incubated with glutamate at a concentration (3 mM) known to produce marked excitation in cerebellar slices¹² was glial swelling in most regions (Fig. 2c) and a band of intense neuronal swelling near the cut edges of the slices. These effects will be described in detail elsewhere¹³. Away from this damage at the cut edges, neurones remained intact but became necrotic (non-selectively) if a higher concentration (10 mM) was applied. The normally just sub-toxic concentration of kainate (3 μ M) did not become toxic to any of the neuronal cell types in the presence of 3 mM glutamate (Fig. 2d).

Three main conclusions can be drawn from these experiments. First, the lowest concentration of kainate (500 μ M) eliciting glutamate release from slices of mouse cerebellum (and other brain areas)^{9,14} is 50–100-fold higher than the concentrations

producing neurotoxic effects against the cells innervated by the parallel fibres. Maximum release of glutamate apparently occurs at 10 mM kainate¹⁴, that is, a concentration 300-fold higher than that required to kill all neuronal cells in cerebellar slices (Fig. 2a). The possibility that the activity of uptake systems imposes a necessity for such high kainate concentrations to be used in order for a glutamate overflow to be detectable is unlikely for several reasons: for example, in the experiments of Ferkany *et al.*^{9,14}, the measured release of amino acids was apparently uncomplicated by reuptake (to judge from the lack of effect of uptake inhibition) and significant glutamate release could be evoked by moderate concentrations of K⁺ ions. Moreover, kainate has been shown to be even less effective in releasing glutamate from synaptosomal preparations¹⁵, in which the influence of such barriers would be substantially reduced. The ability of kainate to release amino acids therefore probably plays no

significant part in its neurotoxic effects. Indeed, there is evidence that the kainate receptors associated with granule cells are more likely to be dendritic than axonal^{11,16} and that the origin of the released glutamate is nonsynaptic rather than synaptic¹⁵.

The second conclusion is that D- α -amino adipate does not attenuate the neurotoxicity of kainate at the concentrations used by Ferkany *et al.*⁹. The interpretation of their data on cyclic GMP stimulation (from which this inference was drawn) is made more difficult because the responses are so small, suggesting that the majority of cells in the slices used in this study were dead, even in the absence of kainate^{10,17}. Third, we could obtain no evidence that the availability of glutamate is a limiting factor in kainate neurotoxicity in the cerebellum. These three lines of evidence seem sufficient to reject the scheme for kainate neurotoxicity proposed by Ferkany *et al.*⁹.

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Identification of presynaptic serotonin autoreceptors using a new ligand: ³H-PAT

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Binding studies with appropriate labelled ligands have revealed the existence of two types of serotonin (5-HT) receptor, 5-HT₁ and 5-HT₂, in the central nervous system of mammals¹. The 5-HT₁ type is characterized by a higher affinity for agonists than for antagonists, whereas the 5-HT₂ type binds preferentially to antagonists. However, neither of these receptor types apparently corresponds to the presynaptic autoreceptor controlling 5-HT release². In an attempt to identify the presynaptic autoreceptor directly, we synthesized the tritiated derivative of 8-hydroxy-2-(di-*n*-propylamino) tetralin (PAT), a new tetralin derivative with potent 5-HT agonist properties³ and carried out binding studies with rat brain membranes. As we report here, in the hippocampus, the properties of ³H-PAT binding sites correspond closely to those of 5-HT₁ sites. In contrast, in the striatum, ³H-PAT binding sites exhibit a subcellular distribution and pharmacological characteristics usually associated with presynaptic autoreceptors. Furthermore, a marked loss of ³H-PAT binding sites occurs in the striatum (but not in the hippocampus) after the selective degeneration of serotonergic fibres in 5,7-hydroxytryptamine (5,7-HT)-treated rats. Conversely, the sprouting of additional 5-HT terminals in the brain stem of adult rats treated at birth with 5,7 HT is associated with an increased density of ³H-PAT binding sites in this region. ³H-PAT thus seems to be a useful ligand for studying the biochemical and pharmacological characteristics of presynaptic autoreceptors in selected regions of rat brain.

³H-PAT was synthesized by the reduction (Na, *n*-butanol) of 1,7-dimethoxynaphthalene into 8-methoxy-2-tetralone, which was subsequently shaken with *N*-diallylamine in ether and reduced with tritium in the presence of Pd/C. The resulting ³H-8-methoxy-2-(di-*n*-propylamino)tetralin was purified using Silicagel column chromatography and then demethylated by hydrobromic acid in acetic acid. The final product, ³H-PAT,

was purified by preparative TLC. The hydrobromide salt of ³H-PAT presently used for binding assays was 99% pure with a specific activity of 105 Ci mmol⁻¹.

The incubation of hippocampal membranes with nanomolar concentrations of ³H-PAT revealed the presence of specific sites from which the ligand could be completely displaced by 10 μ M unlabelled PAT or 5-HT (Fig. 1). Scatchard analysis of the binding data demonstrated only one slope with a *K_d* of ~4 nM. Similar *K_d* values were found using cortical, hypothalamic, brain stem and collicular membranes. In the striatum, the *K_d* was slightly higher than in other brain regions (*K_d* ~ 10 nM).

The regional distribution of ³H-PAT binding sites was highly correlated (*r* = 0.95) with that of ³H-5-HT binding sites⁴ in 10 selected areas. The density of both sites decreased in the following order: hippocampus > septum > entorhinal cortex > frontal cortex > colliculi > hypothalamus $\approx$ raphe area > brain stem $\approx$ spinal cord > cerebellum. Only the striatum stood outside this correlation, ³H-PAT binding in this region being the same level as that found in the brain stem, whereas striatal ³H-5-HT binding was as high as that found in the septum. This phenomenon led us to explore further the characteristics of ³H-PAT binding to striatal membranes compared with those found in other regions, particularly the hippocampus, paying special attention to the pre- or postsynaptic location of these binding sites.

The first set of experiments investigated the subcellular distribution⁵ of ³H-PAT binding in the striatum and the hippocampus and compared it with ³H-imipramine⁶ and ³H-5-HT binding. As expected for a presynaptic marker^{6,7}, ³H-imipramine binding was maximal in fraction M, containing nerve endings; in contrast, ³H-5-HT binding was maximal in the microsomal P fraction, enriched with postsynaptic plasma membranes. ³H-5-PAT binding in the hippocampus was distributed similarly to ³H-5-HT binding, whereas in the striatum, the distribution of specific ³H-PAT binding sites corresponded more closely to that of ³H-imipramine binding sites.

To confirm the post- and presynaptic locations of ³H-PAT-specific binding sites in the hippocampus and striatum, respectively, a second set of experiments was performed using various neurotoxins. The intracerebral injection of 5,7-HT to adult rats induced extensive degeneration of serotonergic fibres as shown by a marked reduction in synaptosomal uptake of ³H-5-HT in both striatum and hippocampus (Table 1). The disappearance of serotonergic terminals was associated with a significant loss of specific ³H-PAT binding sites in the striatum

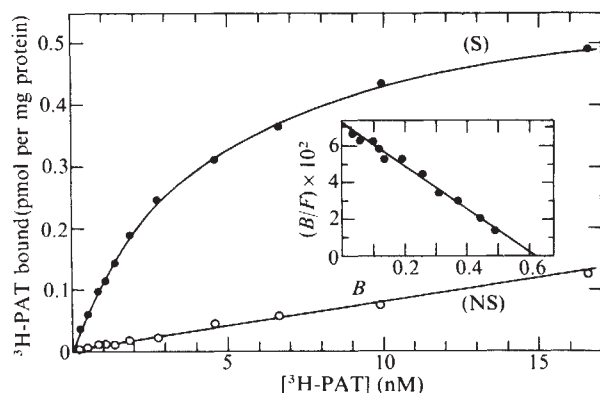


Fig. 1 Binding of ^3H -PAT to crude membranes from the rat hippocampus. Pooled hippocampi from 15 adult male rats were homogenized in 20 vol. (v/w) of 0.05 M Tris-HCl, pH 7.4, using a Polytron PT 10 OD apparatus. Crude hippocampal membranes were then prepared as described previously⁸. Aliquots of the final membrane suspension (corresponding to 0.8–1.0 mg protein) were incubated for 10 min at 37°C in 2 ml (final volume) of 0.05 M Tris-HCl, pH 7.4, containing 0.27–16.6 nM of ^3H -PAT. Other samples were supplemented with 10 μM 5-HT. In these conditions, binding equilibrium was reached within 5 min. Membranes were then collected by filtration through Whatman GF/B filters. After washing with 3 $\times$ 3 ml of ice-cold Tris buffer, filters were dried by heating at 60°C for 1 h and thoroughly mixed with 10 ml of Aquasol (NEN) for radioactivity counting. In the absence of membranes, about 1% of total radioactivity was entrapped within GF/B filters; neither 'cold' PAT nor 5-HT affected this nonspecific binding. Bound and free radioactivity at the end of a 10 min incubation period co-migrated with authentic PAT in two different TLC systems (benzene/tetrahydrofuran 50:50 by vol., and ethylacetate/methanol 95:5 by vol.). $\circ$ (NS), Binding of ^3H -PAT in the presence of 10 μM 5-HT (nonspecific binding). $\bullet$ (S), Total binding minus that persisting in the presence of 10 μM 5-HT (specific binding). Each point is the mean of triplicate determinations. Inset: Scatchard plot of the specific binding of ^3H -PAT to hippocampal membranes. B , ^3H -PAT specifically (S) bound in pmol per mg protein. B/F = bound over free ^3H -PAT in each sample. For this particular experiment, $K_d = 4.29$ nM and $B_{\max} = 0.624$ pmol per mg protein.

but not in the hippocampus. Furthermore, when 5,7-HT was injected in neonates, a reduction of ^3H -5-HT uptake was observed 3 months later in the hippocampus and in the spinal cord but not in the striatum. In the brain stem, a proliferation of additional 5-HT terminals (sprouting phenomenon, see ref. 8) had occurred, as indicated by a marked increase in the $V_{\max}$ of ^3H -5-HT uptake in this region. Except in the hippocampus, the changes in ^3H -PAT binding sites after neonatal 5,7-HT treatment were the same as the changes in the density of presynaptic 5-HT uptake sites. Thus, in the spinal cord, a significant decrease in the $B_{\max}$ of ^3H -PAT binding sites was associated with the degeneration of 5-HT terminals, whereas in the brain stem, an increase in the total number of binding sites was associated with the proliferation of additional 5-HT nerve endings (Table 1).

Because 5,7-HT treatment resulted in the partial disappearance of ^3H -PAT binding sites, notably in the striatum, the possible location of ^3H -PAT binding sites outside serotonergic neurones was examined after the lesion of other neuronal populations. Although 5-HT receptors exist on striatal dopaminergic terminals⁹, they were not involved in the specific binding of ^3H -PAT, as the selective degeneration of catecholaminergic systems resulting from the injection of 6-hydroxydopamine (6 μg) into the medial forebrain bundle did not affect ^3H -PAT binding to striatal membranes. The local injection of kainic acid (2 μg) is known to induce the degeneration of intrinsic neurones in the hippocampus¹⁰ and striatum¹¹, while sparing afferent (serotonergic) fibres to these structures. Two weeks after this treatment, ^3H -PAT binding was markedly reduced in the hippocampus (control rats: $B_{\max} = 603 \pm 54$ fmol per mg protein; kainic acid-treated rats: $B_{\max} = 314.8 \pm$

Table 1 Effects of 5,7-dihydroxytryptamine treatment on ^3H -5-HT synaptosomal uptake and ^3H -PAT binding in various regions

Structure	Treatment	^3H -5-HT uptake $V_{\max}$	^3H -PAT binding $B_{\max}$
Expt I			
Striatum	Control	23.3 $\pm$ 1.7	278.2 $\pm$ 22.1
	5,7-HT	5.4 $\pm$ 0.6* (−76.8%)	101.4 $\pm$ 9.1* (−63.6%)
Hippocampus	Control	18.9 $\pm$ 1.5	612.1 $\pm$ 24.9
	5,7-HT	2.3 $\pm$ 0.2* (−87.8%)	637.9 $\pm$ 33.2 (+4.2%)
Expt II			
Spinal cord	Control	16.5 $\pm$ 1.2	97.8 $\pm$ 15.0
	5,7-HT	2.1 $\pm$ 0.2* (−87.3%)	54.6 $\pm$ 6.5 (−44.2%)
Hippocampus	Control	21.9 $\pm$ 2.2	584.7 $\pm$ 52.6
	5,7-HT	4.0 $\pm$ 0.3* (−81.8%)	617.8 $\pm$ 38.6* (+5.7%)
Brain stem	Control	26.2 $\pm$ 2.8	224.0 $\pm$ 16.4
	5,7-HT	38.6 $\pm$ 3.2* (+47.3%)	319.7 $\pm$ 21.6* (+42.7%)

Expt I: Adult male rats were treated with desipramine (Ciba-Geigy, 25 mg per kg, intraperitoneally) 30 min before receiving an intracerebral injection of 5,7-HT (8 μg in 4 μl of isotonic saline containing 0.01% ascorbic acid) at the following coordinates: A = 2.6 mm, L = 0.4 mm, H = 2.2 mm (see ref. 4). Animals were killed 35 days later and the right (ipsilateral to the injection site) striatum and hippocampus were dissected. Expt II: Rats of either sex were treated with 5,7-HT (100 mg per kg, subcutaneously) on days 1 and 2 after birth. Animals were killed 3 months later and the spinal cord, brain stem and hippocampus dissected. For both experiments, the synaptosomal uptake of ^3H -5-HT was measured⁸ using five different concentrations of the labelled substrate (20–226 nM). Apparent K_m and $V_{\max}$ values were determined by linear regression analysis of double reciprocal plots. K_m values (58–77 nM) were not significantly different between regions in control and 5,7-HT-treated rats. $V_{\max}$ values (in pmol ^3H -5-HT per mg protein, 4 min) are the means $\pm$ s.e.m. of determinations made in three separate experiments using crude synaptosomes prepared from identical structures dissected in five control or 5,7-HT-treated rats. The characteristics of ^3H -PAT binding were determined in the same groups of animals using six to eight different concentrations of the labelled ligand (0.5–12.5 nM). Scatchard plots revealed that the apparent K_d values (from 3.5 nM in the hippocampus to 11.2 nM in the striatum) were not significantly affected by the administration of 5,7-HT. $B_{\max}$ values (in fmol ^3H -PAT per mg protein) are the means $\pm$ s.e.m. of determinations made in 3–8 independent experiments with membranes from identical structures of 10–13 control or 5,7-HT-treated rats.

* $P < 0.05$ compared with the respective control values. The percentage change due to 5,7-HT treatment is indicated in parentheses.

17.4 fmol per mg protein, $n = 4$, $P < 0.05$; no significant change in K_d) but not in the striatum (control rats: $B_{\max} = 259.5 \pm 22.4$ fmol per mg protein; kainic acid-treated rats: $B_{\max} = 230.9 \pm 21.8$ fmol per mg protein, $n = 3$, $P > 0.05$; no significant change in K_d). These data confirmed further that ^3H -PAT bound to postsynaptic sites in the hippocampus. In contrast, ^3H -PAT binding was mostly, if not exclusively, associated with intact presynaptic 5-HT structures in the striatum. The ^3H -PAT binding sites remaining 5 weeks after the intracerebral administration of 5,7-HT (Table 1) could indicate the persistence of membranes of degenerating 5-HT fibres in the striatum. Indeed, membranes of degenerating neurones having intact specific binding sites are still present in the striatum several weeks after the local injection of kainic acid¹².

A final set of experiments was performed to determine whether the presynaptic ^3H -PAT binding sites exhibited the pharmacological properties of autoreceptors. Analyses of the characteristics of presynaptic autoreceptors controlling the release of ^3H -5-HT from slices or synaptosomes^{2,13–20} have shown that these receptors are similar but not identical to 5-HT₁ sites. In particular, differences concerning the relative efficacies

Table 2 Relative binding affinities (pIC_{50} , M) of various compounds for 3H -PAT and 3H -5-HT binding sites in the rat hippocampus and striatum

Drug	pIC_{50}		
	Hippocampus 3H -PAT	Hippocampus 3H -5-HT	Striatum 3H -PAT
Agonists			
PAT	8.52 $\pm$ 0.04	7.86*	7.70 $\pm$ 0.06
5-HT	8.17 $\pm$ 0.12	8.0 $\pm$ 0.6†	7.33 $\pm$ 0.13
RU-24969	8.01 $\pm$ 0.08	7.69*	6.04 $\pm$ 0.04
Bufotenine	7.67 $\pm$ 0.07	7.1 $\pm$ 0.2†	5.99 $\pm$ 0.11
5-M-N, N-DMT	7.40 $\pm$ 0.03	7.66*	6.54 $\pm$ 0.14
α -M-5-HT	6.80 $\pm$ 0.09	—	6.30 $\pm$ 0.07
5-MT	6.54 $\pm$ 0.12	—	4.40 $\pm$ 0.05
Tryptamine	5.43 $\pm$ 0.11	6.6 $\pm$ 0.2†	4.63 $\pm$ 0.06
Antagonists			
Metergoline	7.46 $\pm$ 0.25	7.6 $\pm$ 0.2†	6.61 $\pm$ 0.05 (1)
Methysergide	7.15 $\pm$ 0.09	6.9 $\pm$ 0.01†	5.33 $\pm$ 0.14 (4)
Methiothepin	7.05 $\pm$ 0.09	7.1 $\pm$ 0.4†	5.94 $\pm$ 0.09 (2)
Cyproheptadine	6.01 $\pm$ 0.15	6.1 $\pm$ 0.1†	5.28 $\pm$ 0.03 (5)
Cinanserin	5.96 $\pm$ 0.09	5.4 $\pm$ 0.1†	4.94 $\pm$ 0.10 (7)
Pizotifen	5.66 $\pm$ 0.09	5.7 $\pm$ 0.2†	5.21 $\pm$ 0.08 (6)
Quipazine	5.47 $\pm$ 0.10	5.95*	5.93 $\pm$ 0.04 (3)
Ketanserin	5.38 $\pm$ 0.07	—	4.87 $\pm$ 0.03 (8)
Other compounds			
Buspirone	7.53 $\pm$ 0.05	—	6.05 $\pm$ 0.18
Yohimbine	5.88 $\pm$ 0.07	—	5.81 $\pm$ 0.06
Haloperidol	5.45 $\pm$ 0.07	5.0 $\pm$ 0.2†	5.38 $\pm$ 0.06
Dopamine	4.77 $\pm$ 0.15	4.6 $\pm$ 0.5†	3.29 $\pm$ 0.11
Promethazine	4.68 $\pm$ 0.06	—	4.24 $\pm$ 0.06
Imipramine	4.66 $\pm$ 0.06	—	4.76 $\pm$ 0.04
Benzotropine	4.35 $\pm$ 0.05	—	4.42 $\pm$ 0.04
Noradrenaline	3.77 $\pm$ 0.15	3.6 $\pm$ 0.2†	3.26 $\pm$ 0.04

Hippocampal or striatal membranes (0.8–1.0 mg protein) were incubated with 1 nM 3H -PAT and at least six different concentrations of a given drug in conditions described in Fig. 1 legend. pIC_{50} is equal to $-\log$ of the drug concentration (M) producing half-inhibition of the specific binding of 3H -PAT. Each value is the mean $\pm$ s.e.m. of at least three independent determinations. The order of potency of antagonists for displacing 3H -PAT specifically bound to striatal membranes is indicated in parentheses. Calculations of the correlation coefficients between pIC_{50} values gave: $r_1 = 0.857$ between all pIC_{50} values against 3H -PAT binding to hippocampal and striatal membranes. $r_2 = 0.942$ between all pIC_{50} values against 3H -PAT and 3H -5-HT binding to hippocampal membranes. $r_3 = 0.842$ between pIC_{50} values of 5-HT agonists against 3H -PAT binding to hippocampal and striatal membranes. $r_4 = 0.622$ between pIC_{50} values of 5-HT antagonists against 3H -PAT binding to hippocampal and striatal membranes. RU-24969 = 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole. 5-M-N, N-DMT = 5-methoxy-*N,N*-dimethyltryptamine. α -M-5-HT = α -methylserotonin. 5-MT = 5-methoxytryptamine.

* From ref. 23.

† From ref. 24.

of 5-HT antagonists to block either autoreceptors or 5-HT₁ sites have been clearly demonstrated^{2,14–18}. It was therefore of interest to analyse the pharmacological properties of 3H -PAT binding sites in two different regions thought to contain preferentially postsynaptic (hippocampus) or presynaptic (striatum) 3H -PAT binding sites.

5-HT agonists were generally much more potent than antagonists in their ability to displace 3H -PAT from specific hippocampal binding sites. pIC_{50} values for the displacement of 3H -PAT binding were quite similar to those using 3H -5-HT ($r = 0.942$), confirming that 3H -PAT probably binds to 5-HT₁ sites in the hippocampus (Table 2). In addition, as previously shown for 5-HT₁ sites²¹, hippocampal 3H -PAT binding sites were affected by guanine nucleotides and divalent cations: GTP decreased the affinity of agonists for 3H -PAT and 3H -5-HT binding sites whereas divalent cations, particularly Mn^{2+} , had the opposite effect. In contrast, striatal 3H -PAT binding was unaffected by guanine nucleotides, and Mn^{2+} (1 mM) markedly decreased the B_{max} (–60%) without altering the affinity for the ligand. These properties confirmed that striatal 3H -PAT binding

sites were different from 5-HT₁ sites. Striatal 3H -PAT binding sites must, however, be considered as 5-HT-related sites because 5-HT was at least 2×10^4 times more active than dopamine or noradrenaline in its ability to displace 3H -PAT bound to striatal membranes (Table 2). Furthermore, the presynaptic sites in the striatum were clearly distinct from the 5-HT re-uptake sites, because imipramine only poorly inhibited 3H -PAT binding (Table 2).

For both agonists and antagonists, pIC_{50} values determined on striatal 3H -PAT binding were generally lower than those determined using hippocampal membranes (Table 2). However, the order of potency of agonists was similar in the two brain regions. One exception concerned 5-methoxy-*N,N*-dimethyltryptamine, which was more active than bufotenine on striatal 3H -PAT binding but less active on hippocampal 3H -PAT binding (Table 2). This discrepancy deserved some attention because the 5-HT autoreceptor controlling 5-HT release also has a higher affinity for 5-methoxy-*N,N*-dimethyltryptamine than for bufotenine²⁰. The possible identity between striatal 3H -PAT binding sites and 5-HT autoreceptors was further supported when considering the relative efficacies of 5-HT antagonists. For these drugs, the order of potency on 3H -PAT binding in the hippocampus was quite different from that in the striatum. Interestingly, the three most potent antagonists in the striatum, metergoline, methiothepin and quipazine, are also the most effective compounds in preventing the inhibitory effect of 5-HT on its own release via the stimulation of autoreceptors^{2,15,19}.

In conclusion, 3H -PAT bound to both postsynaptic and presynaptic 5-HT sites in brain. In the hippocampus, the pharmacological profile of these sites closely resembled those of postsynaptic 5-HT₁ receptors. In the striatum, however, 5-HT₁ receptors did not seem to be labelled by 3H -PAT, indicating either that these sites might not be identical from one brain region to another, or instead correspond to an heterogeneous population of sites²². The specific sites labelled by 3H -PAT in the striatum were located presynaptically and exhibited at least some of the pharmacological properties expected for the presynaptic 5-HT autoreceptors. Thus, 3H -PAT can be considered as a promising ligand for exploring more directly the biochemical and pharmacological properties of these autoreceptors.

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A VIP-containing system concentrated in the lumbosacral region of human spinal cord

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Neuropeptides were first localized in the human spinal cord by immunocytochemistry¹ and substance P has been shown, by the same method, to be reduced ipsilaterally in the dorsal horn after limb amputation² and bilaterally in the Riley-Day syndrome³. Several neuropeptides increasingly fulfil the criteria to establish them as neurotransmitters or neuromodulators⁴, and they may also have trophic actions in the spinal cord⁵. Using radioimmunoassay and immunocytochemistry, we present here for the first time a quantitative regional distribution and localization of vasoactive intestinal polypeptide (VIP), substance P, somatostatin, bombesin and cholecystokinin (CCK-8) in normal postmortem human spinal cord. A comparison of the distribution of these peptides reveals an exceptional pattern for VIP, with relatively much higher levels in the lumbosacral region. Immunocytochemical analysis shows a distinctive distribution of VIP-containing fibres and terminals at the lumbosacral segments. This VIP-containing system may have an important role in the spinal control of urogenital function in man.

Human spinal cord specimens were collected post mortem from eight adults (six males, two females; mean age 60 yr, range 48–83 yr) with no evidence of nervous system pathology. All cadavers were stored at 4 °C within 2 h of death, and the spinal cords removed within 24 h, a period generally established for stability of neuropeptides in postmortem specimens⁶. For radioimmunoassay, transverse slices (0.5 cm thick) from cervical, thoracic, lumbar and sacral regions were divided into ventral and dorsal regions by section through the central canal, and stored in liquid nitrogen. Peptides were extracted in boiling water (for CCK-8) and 0.5 M acetic acid (all other peptides). Duplicate 10- μ l aliquots of each extract were assayed by specific radioimmunoassays⁷. For immunocytochemistry, slices were fixed in 0.4% *p*-benzoquinone in phosphate-buffered saline (PBS) for 7–12 h, then stored at 4 °C in PBS with 0.7% sucrose and 0.1% sodium azide. The staining procedure was a modification of Sternberger's peroxidase–antiperoxidase technique⁸.

The regional concentrations of peptides are shown in Table 1. In general, peptide concentrations increased caudally: this is

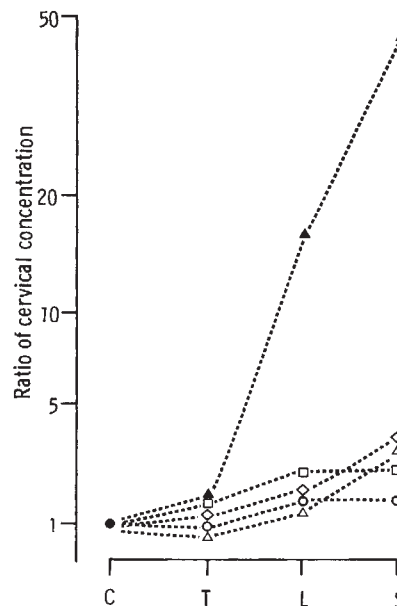


Fig. 1 Dorsal regional concentrations of substance P ($\diamond$), VIP ($\blacktriangle$), somatostatin ($\circ$), bombesin ($\square$) and CCK ($\triangle$) as ratios of their dorsal cervical concentrations. C, cervical; T, thoracic; L, lumbar; S, sacral.

at least partly due to a caudal decrease in the proportion of white matter, which has a lower peptide content. Substance P and somatostatin levels were higher in dorsal than ventral cord (all differences were significant at $P < 0.05$, Student's paired *t*-test), but bombesin and CCK-8 levels did not show such a difference. CCK-8 levels appeared higher in the ventral cord. The pattern of VIP distribution was exceptional (Fig. 1): the levels of VIP were extremely low in the cervical and thoracic region whereas the lumbar and especially the sacral regions showed much higher levels, with dorsal concentrations exceeding ventral concentrations ($P < 0.05$).

Figure 2A and B illustrates regional immunocytochemical localization of substance P and VIP terminals and fibres, respectively. As has been described previously, substance P was mainly localized to the substantia gelatinosa (Fig. 3A), Lissauer's tract, around the central canal, in the dorsolateral white matter, the intermediolateral columns and the ventral horn. Somatostatin showed a similar distribution. The CCK-8-like immunoreactive fibres and terminals were also seen in these regions: they were numerous in the ventral horn. In contrast, there were few VIP-containing fibres in cervical and thoracic regions, and they were seen only as small bundles in Lissauer's tract and the most dorsal portion of the substantia gelatinosa. VIP-containing fibres were more widespread in the dorsolateral lumbar cord and became abundant in the sacral cord, in the dorsolateral grey-

Table 1 Regional concentrations of neuropeptides

	Cervical		Thoracic		Lumbar		Sacral	
	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal
Substance P	23.0 $\pm$ 3.5	39.8 $\pm$ 6.2	20.9 $\pm$ 2.5	38.0 $\pm$ 7.0	32.5 $\pm$ 7.2	48.8 $\pm$ 14.7	99.2 $\pm$ 21.0	158.0 $\pm$ 29.0
Bombesin	0.47 $\pm$ 0.10	0.44 $\pm$ 0.06	0.49 $\pm$ 0.10	0.45 $\pm$ 0.14	0.68 $\pm$ 0.18	0.86 $\pm$ 0.20	1.09 $\pm$ 0.40	1.13 $\pm$ 0.41
CCK-8	9.3 $\pm$ 1.3	6.2 $\pm$ 0.7	5.8 $\pm$ 1.4	3.4 $\pm$ 1.1	10.4 $\pm$ 1.4	6.5 $\pm$ 0.9	30.7 $\pm$ 1.8	23.5 $\pm$ 4.6
Somatostatin	6.05 $\pm$ 0.6	12.8 $\pm$ 1.9	4.9 $\pm$ 0.8	14.3 $\pm$ 1.9	9.4 $\pm$ 0.8	22.0 $\pm$ 2.3	15.9 $\pm$ 2.1	24.7 $\pm$ 1.7
VIP	0.63 $\pm$ 0.06	0.64 $\pm$ 0.19	0.86 $\pm$ 0.18	0.90 $\pm$ 0.28	0.82 $\pm$ 0.20	10.75 $\pm$ 2.6	7.7 $\pm$ 1.9	30.0 $\pm$ 6.2

Values are pmol g⁻¹ (mean $\pm$ s.e.m.). Gel filtration of extracts on Sephadex G-50 and G-25 showed that the peptide immunoreactivities were eluted with K_{av} values similar to those of their respective pure peptide standards. $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e is the elution volume, V_o the void volume, and V_t the total bed volume available for diffusion of solutes. Somatostatin immunoreactivity emerged as two peaks corresponding to somatostatin-14 (expected G-50 K_{av} = 0.87, observed K_{av} = 0.84) and somatostatin-28 (expected K_{av} 0.60, observed 0.58) with a preponderance of somatostatin-14. Bombesin-like immunoreactivity also gave two peaks in similar amounts which coeluted with porcine gastrin-releasing peptide (expected G-50 K_{av} 0.54, observed 0.54) and amphibian bombesin (expected K_{av} 0.76, observed 0.78). Substance P (expected G-25 K_{av} 0.33, observed 0.34), VIP (expected G-50 K_{av} 0.55, observed 0.54) and CCK-8 (expected G-25 K_{av} 1.03, observed 1.02) emerged as single peaks.

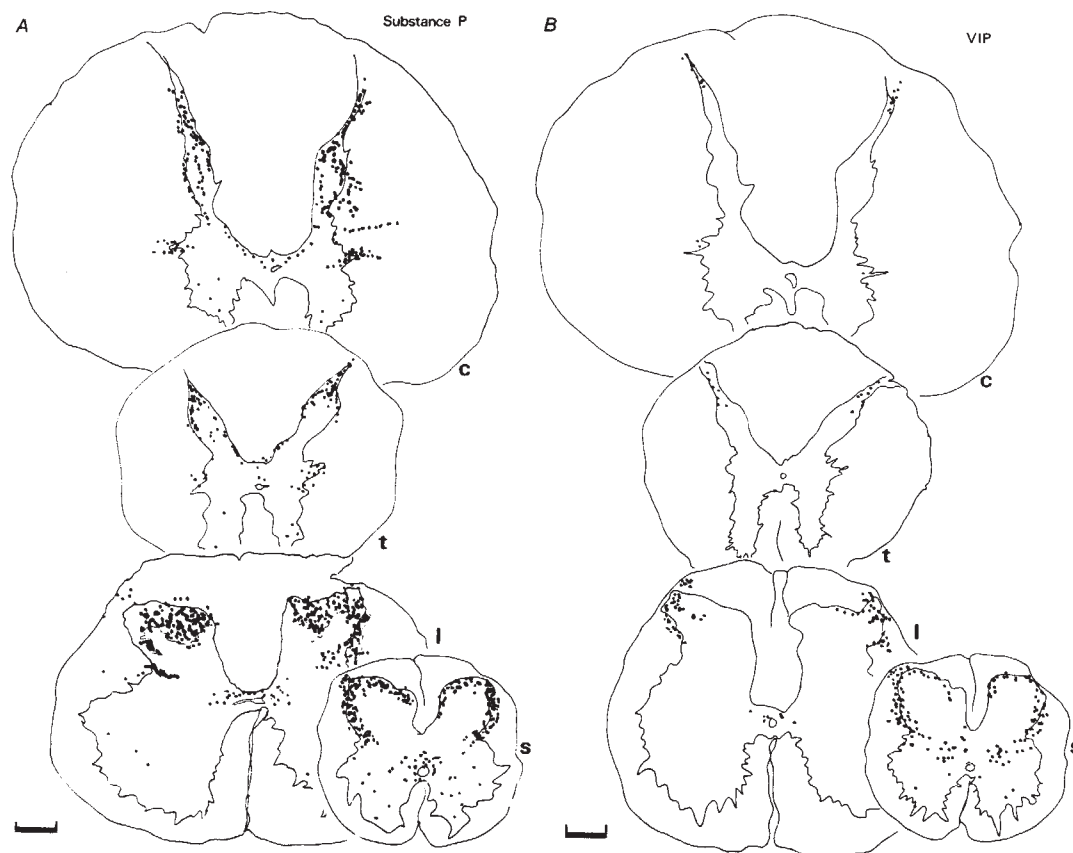


Fig. 2 Distribution of substance P (A) and VIP (B) fibres and terminals in the cervical (c), thoracic (t), lumbar (l) and sacral (s) human spinal cord. The maps were constructed using camera lucida; scale bar, 1 mm. Substance P-immunoreactive fibres were more numerous in the substantia gelatinosa, around the central canal and in the intermediolateral cell column of the thoracic segments. VIP-containing fibres were less frequently observed but the distribution followed a similar pattern. There was a remarkable increase of fibres in the dorsal horn of lumbar and especially sacral segments.

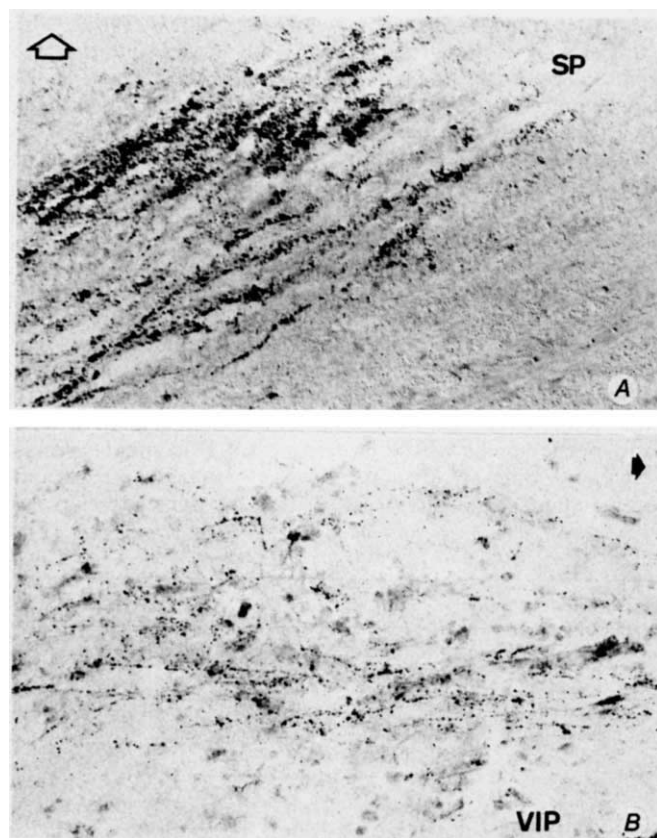


Fig. 3 A, Substance P (SP)-immunoreactive fibres in the substantia gelatinosa of the thoracic spinal cord. Arrow points dorsally. $\times 140$. B, VIP-containing fibres in the lateral dorsal horn of the sacral spinal cord. Numerous fine fibres with irregular varicosities are apparent at this level of the spinal cord. Arrow points dorsally. $\times 140$.

white matter border (Fig. 3B) and between the intermediolateral column and central canal.

Further studies are needed to establish the origin and function of VIP-containing fibres and terminals in the human lumbosacral cord. The distribution of VIP-immunoreactive fibres suggests that they could originate from primary afferent nerves, spinal interneurons and descending supraspinal tracts. It is likely that at least some of the VIP-like immunoreactivity is in primary afferent nerves from pelvic visceral organs, for the following reasons. The regional distribution of VIP and the pattern of terminals and fibres showing VIP-like immunoreactivity in the cat spinal cord are indistinguishable from those in human cord; VIP-containing fibres have been observed in abundance in the sacral dorsal roots of the cat and significant levels of VIP have been found in lumbosacral dorsal roots in humans (our unpublished observations). Further, there is a striking similarity between the immunocytochemical localization of VIP in cat and human lumbosacral cord and the central termination of fibres from the pelvic nerve of the cat, as labelled by retrograde horseradish peroxidase tracing⁹. The pelvic nerve terminals in the cat spinal cord are mostly collaterals of axons in Lissauer's tract that course laterally and medially around the dorsal horn, and are located in close proximity to preganglionic cell bodies and dendrites. These pelvic afferents share other features with the distribution of VIP-immunoreactive terminals—there is a periodic or metameric collection of afferents in the sacral cord, and they terminate rostrally and caudally beyond the limits of the sacral parasympathetic neurones, reflecting the integration of somatic and autonomic inputs in pelvic visceral function.

Although the lateral projection from Lissauer's tract is a massive pathway in the sacral cord, the failure to identify it in several dorsal root degeneration studies has been attributed to the lack of sensitivity of silver impregnation methods in demonstrating fine afferents. Nevertheless, there is evidence in monkeys that visceral afferents do not synapse directly with autonomic efferent neurones¹⁰; visceral reflexes are mediated via interneurons and may involve supraspinal pathways¹¹. Loss of supraspinal influences on micturition reflexes may be observed

after lesions that affect lateral white matter pathways cephalic to the human lumbosacral cord¹²: these include autonomic reflex activity following a peripheral sensory stimulus which would not previously have been adequate to initiate the reflex¹³. The distribution of VIP in lumbosacral cord is suitably located to mediate supraspinal modulation of visceral reflexes, and to serve as interneurons between their afferent and efferent arcs. Whatever its origin, this VIP-containing system probably plays an important part in the spinal control of urogenital function in man.

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Sustained rise in ACh sensitivity of a sympathetic ganglion cell induced by postsynaptic electrical activities

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Long-term alteration in synaptic efficacy found in several neurones of both vertebrates and invertebrates has been suggested as an important mechanism for learning and memory^{1–3}. In bullfrog sympathetic ganglia, acetylcholine (ACh) release from presynaptic nerve terminals is potentiated for a long time by adrenaline through a cyclic AMP system^{4,5}. We report here a new form of mechanism for long-term synaptic potentiation in sympathetic ganglia, which occurs postsynaptically in a Ca^{2+} -dependent manner. Our results suggest that Ca^{2+} entry into a ganglion cell during repeated action potentials initiates a long-lasting mechanism for the enhancement of a nicotinic ACh action on the subsynaptic membrane. This, as well as the presynaptic mechanism^{4–6}, may contribute to neuronal plasticity in the peripheral autonomic nervous system.

Antidromic stimulation of a frog preganglionic nerve trunk (applied in normal Ringer's solution) potentiated the amplitude of the fast excitatory postsynaptic potential (e.p.s.p., 199 ± 18 ($\pm$ s.e.), $n = 5$, of the control at 70 min after the stimulation) recorded in a low Ca^{2+} -high Mg^{2+} solution (Fig. 1B, C), with an increase in the decay half-time ($171 \pm 17\%$, $n = 5$; Fig. 1B). This potentiation seemed to level off about 2 h after the stimulation and showed no tendency towards recovery for at least 3 h. Quantal analysis revealed a significant increase in quantal size ($167 \pm 21\%$, $n = 5$, at 70 min after stimulation) during the potentiation, with a small rise in the quantal content ($124 \pm 16\%$, $n = 5$, $P > 0.1$), indicating that a postsynaptic mechanism predominates in the potentiation of the fast e.p.s.p.

Potentiation of the fast e.p.s.p. was indeed the result of antidromic stimulation. Perfusion of the ganglion with normal Ringer's for 10 min without stimulation only slightly increased the quantal size ($111 \pm 6\%$, $n = 6$, $P > 0.1$, 70 min after wash) and quantal content ($126 \pm 19\%$, $n = 6$, $P > 0.1$). Moreover, when the antidromic tetanus was applied without altering the

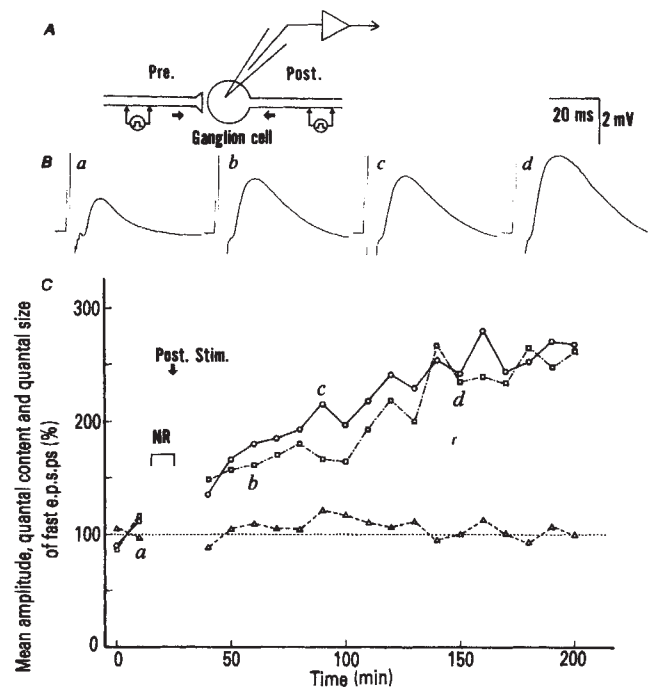


Fig. 1 A, Stimulating and recording arrangement. The fast e.p.s.p. was induced by stimulating a preganglionic nerve trunk (left). Antidromic stimulations (20 Hz pulses for 5 s) were applied to a postganglionic nerve trunk (right). B, Effects of antidromic stimulation in normal Ringer's on the fast e.p.s.p. recorded in a low Ca^{2+} -high Mg^{2+} solution. A low Ca^{2+} -high Mg^{2+} solution was changed to normal Ringer's (NR) for 10 min before stimulation and returned to a low Ca^{2+} -high Mg^{2+} solution immediately after stimulation. All records were electronic averages of 200 fast e.p.s.p.s. C, Time course of changes in the mean amplitude ($\circ$), quantal content (Δ) and quantal size ($\square$) of fast e.p.s.p.s. before and after stimulation. The quantal content was calculated by both variance and failure methods²² and the average taken. The values of quantal contents obtained by both methods agreed fairly well in a range 0.2–3. The quantal size was computed by dividing the mean amplitude by the quantal content. Values for each parameter (ordinate) were normalized to those before stimulation. Symbols a, b, c and d in C correspond to those in B. Note that the potentiation of the fast e.p.s.p. by an antidromic tetanus was not seen in rat superior cervical ganglia¹⁹. Such a difference may be due to a species difference.

Methods: Isolated ninth or tenth lumbar sympathetic ganglia of bullfrogs (*Rana catesbeiana*: B-type neurones) were studied using a conventional intracellular recording technique²³. Microelectrodes were filled with 3 M KCl (tip resistance 30–100 M Ω). The fast e.p.s.p. was induced at 0.33 Hz in a low Ca^{2+} -high Mg^{2+} solution (Ca^{2+} : 0.5–0.9 mM; Mg^{2+} : 5.4–7.7 mM) and its quantal contents and sizes were calculated from 100–200 fast e.p.s.p.s as reported previously, using a microcomputer⁵. The fast e.p.s.p. was also recorded in normal Ringer's containing (+)tubocurarine (30–50 μ M). The rate and duration of tetanic antidromic or orthodromic stimulations (20 Hz, 5 s) were kept constant in this study. Experiments were carried out at room temperature (20–24 °C).

perfusion solution, it potentiated the amplitude of the fast e.p.s.p. recorded in normal Ringer's containing (+)tubocurarine ($139 \pm 11\%$, $n = 5$, 70 min after tetanus), although the e.p.s.p. amplitude decreased for several minutes immediately after the tetanus ($85 \pm 6\%$, $n = 6$, at 5 min)⁷.

An antidromic tetanus also potentiated the amplitude of a nicotinic ACh potential induced by iontophoretic application of ACh in normal Ringer's ($197 \pm 38\%$, $n = 4$, 1 h after stimulation), with a constant peak time (Fig. 2A). As the membrane resistance was unchanged throughout the experiment (Fig. 2B), this increase in the ACh potential was due to enhancement of the nicotinic action of ACh on the postsynaptic membrane. This, together with the increased fast e.p.s.p. amplitude in normal Ringer's with (+)tubocurarine, indicates that the postsynaptic

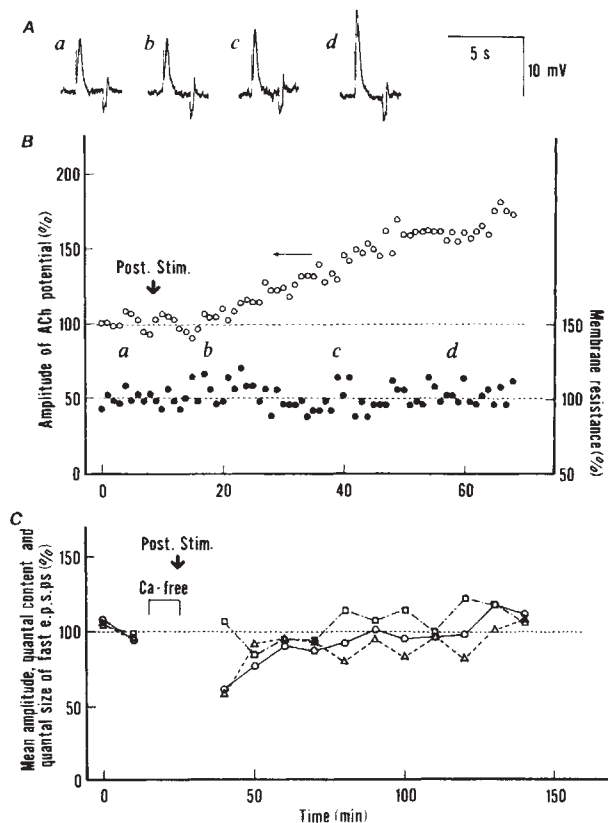


Fig. 2 A, Effects of antidromic stimulation on nicotinic ACh potentials induced by iontophoretic application of ACh and electronic potentials generated by a hyperpolarizing current pulse (0.1 nA, 250 ms) in normal Ringer's. B, Time course of changes in the amplitude of nicotinic ACh potential (○) and the membrane resistance (●) before and after stimulation. Each data point is the average of six consecutive values which were normalized to those before tetanus. The ordinate on the left-hand side represents changes in the amplitude of ACh potential, while that on the right-hand side indicates changes in membrane resistance. Symbols a, b, c and d in B correspond to those in A. C, Effects of antidromic stimulations in a Ca²⁺-free solution on the fast e.p.s.ps recorded in a low Ca²⁺-high Mg²⁺ solution. The mean amplitude (○), quantal content (△) and quantal size (□) of fast e.p.s.ps were plotted in normalized scales to those before tetanus in ordinate. **Methods:** The nicotinic or muscarinic ACh potentials were induced by iontophoretic application of ACh (2 M ACh-Cl, tip resistance 20–50 MΩ) to the ganglion cell membrane at a rate of 0.1 Hz (nicotinic potentials) or 0.0167 Hz (muscarinic potentials). A Ca²⁺-free solution contained 10.8 mM MgCl₂, the tonicity of which was adjusted by changing the concentration of NaCl.

potentiation occurs at the normal Ca²⁺ concentration. In contrast to the nicotinic action, the muscarinic action of ACh which generates a slow e.p.s.p. was not affected by tetanic antidromic stimulation (in two cells, 93 and 101%, 1 h after tetanus). (However, in mammalian ganglia, a preganglionic tetanus caused long-term potentiation of the slow e.p.s.p. only⁸.)

As Ca²⁺ is a charge carrier for the action potential in the bullfrog sympathetic ganglion cell^{9,10}, we considered that the activity-dependent postsynaptic potentiation may have been due to Ca²⁺ entry into the cell during repeated action potentials. This we found to be the case. Antidromic stimulation in a Ca²⁺-free solution had almost no effect on the quantal size (101 ± 9%, n = 5, 70 min after tetanus) or decay half-time (110 ± 13%, n = 5) of the fast e.p.s.p. (Fig. 2C). Thus, a transient rise in the intracellular Ca²⁺ concentration during repeated action potentials would initiate the enhancement of the nicotinic action of ACh at the subsynaptic membrane. Note that this postsynaptic long-term potentiation (p.l.t.p.) also occurred following the physiological activation of ganglion cells, as indicated by increases in the quantal size of the fast e.p.s.p.

(142 ± 21%, n = 4, 70 min) and the nicotinic ACh potential amplitude (in two cells, 151 and 158% at 30 min) after the orthodromic tetanus in normal Ringer's.

There are several possible mechanisms for the p.l.t.p. First, a rise in the Ca²⁺ concentration in the cell soma due to its repetitive activities may release some form of neurotransmitter (for example, adrenaline¹¹, luteinizing hormone releasing hormone (LHRH), ATP)¹², which acts back on the soma itself to raise the efficacy of the nicotinic action of ACh. However, this seems highly unlikely in the case of adrenaline, due to the unchanged quantal size of the fast e.p.s.p. after adrenaline (10 μM) pretreatment (85 ± 5%, n = 7, at 1 h after wash)^{4,5}. LHRH and ATP also seem unlikely candidates, because they did not enhance the nicotinic responses of the ganglion cell^{13,14}. However, such a mechanism involving unidentified transmitters cannot be ruled out. Even if this is so, the long-lasting action of such a putative substance on the ganglion cell may not be mediated by an increase in endogenous cyclic AMP or cyclic GMP⁴.

Second, Ca²⁺ entering the cell may directly activate a sequence of biochemical steps responsible for the p.l.t.p. Possible evidence for this lies in the existence of calmodulin (an important mediator of various intracellular Ca²⁺ actions) at the postsynaptic membrane of central neurones¹⁵ and the finding that phosphorylation of the nicotinic cholinergic receptor occurs in the presence of Ca²⁺ and calmodulin¹⁶.

Third, the increased sensitivity of the subsynaptic membrane to ACh might be caused by an increase in the number of the nicotinic cholinergic receptors due to enhanced receptor synthesis¹⁷ or the unmasking of 'silent' receptors¹⁸. Whatever the mechanism, it is apparent that the p.l.t.p. must involve a mechanism which develops gradually after a brief period of Ca²⁺ influx and lasts for a long time.

The p.l.t.p. found in bullfrog sympathetic ganglia in the present study is a new form of functional synaptic plasticity distinct not only from those induced by a catecholamine-cyclic AMP system in bullfrog^{4,5} and rabbit⁸ ganglia, but also from those of central and other peripheral neurones^{1,3,19}. For instance, long-term potentiation in the hippocampus is accounted for by an increased release of transmitter²⁰, although postsynaptic mechanisms cannot be ruled out^{1,18}. Also, the long-lasting enhancement in synaptic transmission induced by serotonin in *Aplysia* neurones has been analysed in detail³ and been found to be presynaptic in origin. Moreover, although a postsynaptic long-term potentiation was found in motor cortical neurones of cat²¹, it was mostly explained by an accompanying increase in the cell input resistance²¹.

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ATP-regulated K^+ channels in cardiac muscle

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An outward current of unknown nature increases significantly when cardiac cells are treated with cyanide or subjected to hypoxia¹⁻⁴, and decreases on intracellular injection of ATP⁵. We report here that application of the patch-clamp technique to CN-treated mammalian heart cells reveals specific K^+ channels which are depressed by intracellular ATP (ATP_i) at levels greater than 1 mM. For these channels, conductance in the outward direction is much larger than the inward rectifier K^+ channel which is insensitive to ATP. AMP had no effect on the ATP-sensitive K^+ channel, and ADP was less effective than ATP. Thus, the ATP-sensitive K^+ channel seems to be important for regulation of cellular energy metabolism in the control of membrane excitability.

The patch-clamp technique⁶ was applied to ventricular and atrial cells isolated from guinea pig or rabbit heart with collagenase⁷. Intracellular ATP synthesis was depressed by superfusing the cells with glucose-free Tyrode's solution containing 5.4 mM CN. Treatment with cyanide for 20–60 min evoked a marked single-channel current (Fig. 1A) which was not evident before the treatment (see, for example, Fig. 2A). Figure 1B shows the current-voltage relation of the channel, the slope of which is almost linear and gives a conductance of 21 pS. The

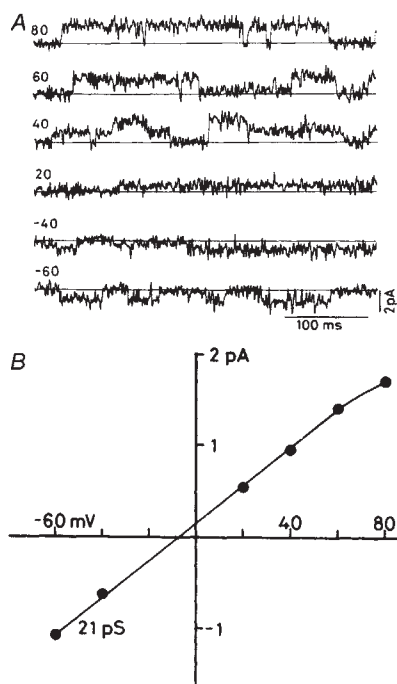


Fig. 1 A, Patch current recordings in the cell-attached mode from a ventricular cell after perfusion with glucose-free, 5.4 mM CN-Tyrode's for 20 min. The electrode contained normal Tyrode's solution (5.4 mM K^+). Deviation of the membrane potential from the resting potential is indicated on the left of each trace. No significant current was observed in the control before CN treatment. The traces were displayed through a low-pass filter with a cut-off frequency of 800 Hz. The relatively large amplitude of the background noise is due to continuous perfusion in the recording chamber. Currents flowing from the internal to the external side are displayed upwards. B, Unit amplitude of the current (ordinate) plotted against deviation of the membrane potential (abscissa) from the resting potential.

reversal potential was ~ 10 mV negative to the resting potential, which was -82 ± 2 mV (mean $\pm$ s.d.) as measured in 16 cells using the patch electrode (whole cell recordings) in CN-Tyrode's. That the reversal potential was about the same as the K^+ electrode potential (E_K) suggested that this channel is selective to K^+ ions.

If the appearance of the K^+ channel is directly linked to a decrease in ATP_i, the inside-out patch membrane bathed in ATP-free solution should open the K^+ channels. To facilitate isolation of the inside-out patch, ventricular cells were superfused with 140 mM K^+ , 1 mM EGTA solution. In this solution, inward single-channel currents were observed with hyperpolarization, but with depolarization no significant current was recorded in the cell-attached recording mode (Fig. 2A, left-hand column). When this patch was isolated and the intracellular surface of the membrane exposed to the bath solution, a marked outward current was apparent (right-hand column). For a transmembrane potential of -80 mV, two different unit amplitudes of the current were apparent. One of these seems identical to the events recorded in the cell-attached mode (inward rectifier K^+ channel), while the other can be identified by larger amplitude and current fluctuations during channel

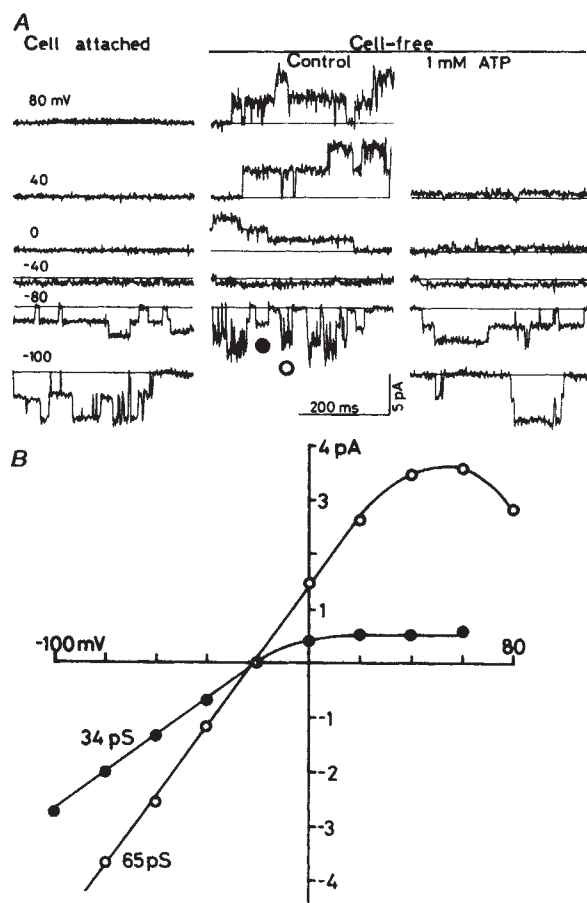


Fig. 2 A, Continuous recordings from the same patch in the cell-attached mode (left-hand column), and inside-out mode before (middle) and after (right) applying 1 mM ATP to the inner surface of the membrane. ●, Inward rectifier channel; ○, ATP-sensitive K^+ channels at -80 mV. B, Current voltage relations plotted for each channel in A. ●, Inward rectifier K^+ channel; ○, the ATP-sensitive K^+ channel. The electrode solution contained 50 mM K^+ which was replaced with equimolar Na^+ in the control Tyrode's. The membrane potentials are indicated on the left of each trace in A. The external side of the membrane was taken as reference. The resting potential of the cell was assumed to be zero in 140 mM KCl solution. The traces in cell-attached recordings are typical for the inward rectifier K^+ channels, while in the cell-free patch an additional channel of larger current amplitude appeared. The larger events disappeared during application of 1 mM ATP.

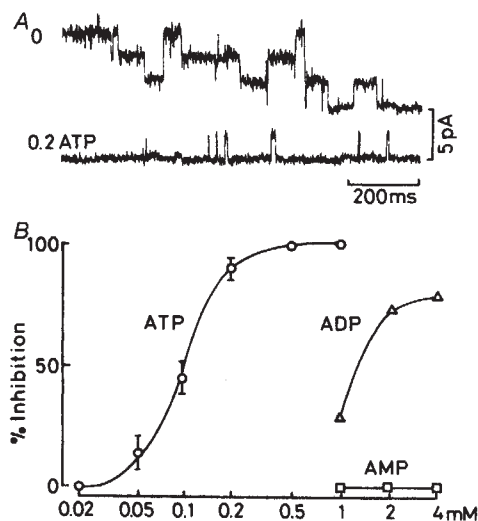


Fig. 3 Effects of ATP, ADP and AMP at various concentrations. The potential of the inside-out patch membrane was maintained at +20 mV, where the single-channel current is outward-going (upward deflections in current recordings in A). The electrode solution contained 50 mM K⁺ (see Fig. 2). A, The upper trace was obtained in the absence and the lower trace in the presence of 0.2 mM ATP at the inner surface of the membrane. ATP shortened the open time of the channel without affecting the unitary amplitude of the current. The overlap of up to three open channels in the upper trace was rarely observed in the presence of ATP. B, Shows the decrease in the average current through the ATP-sensitive K⁺ channels in the presence of ATP, ADP and AMP. The average current was calculated by integrating current flow during the channel openings and dividing the integral by the total time of the sample (generally 20–30 s of data at each ATP concentration). Average current is given by NPI , where N is the number of channels in the patch, P the probability that a single channel is open and I the amplitude of the single-channel current. Values are mean $\pm$ s.e. of six ATP experiments and the average of three ADP or AMP experiments.

opening (ATP-sensitive K⁺ channel). When 1 mM ATP was applied to the inner surface of the membrane, the single-channel current of larger amplitude was selectively suppressed within 10 s (Fig. 2A, right-hand column), this effect was reversible. The current-voltage relationship in Fig. 2B clearly demonstrates the two different classes of K⁺ channels in the patch membrane. The current with a conductance of 34 pS has strong inward rectification, indicating that the channel of small conductance is the inward rectifier K⁺ channel, as has been identified in the ventricular cell and in other excitable cells^{8–10}. The conductance of the ATP-sensitive K⁺ channel, was respectively, 20, 63 and 80 pS for 5.4, 50 and 100 mM K⁺ at the outer surface of the membrane. Such an ATP-sensitive K⁺ channel was also observed in the atrial cells.

To determine the ATP concentration critical for channel opening, we constructed a dose-response curve. When various ATP concentrations were applied to the inner surface of the inside-out patch membrane, the probability of channel opening was progressively reduced by increasing ATP from 0.05 to 1 mM. At intermediate ATP concentrations, the open time was obviously shortened without affecting the unit amplitude (Fig. 3A). The degree of inhibition, plotted on the ordinate of Fig. 3B, was measured as a decrease in the time-averaged current through the ATP-sensitive channel. Compared with ATP, ADP was less potent by one order of magnitude in depressing the channel and AMP had no effect.

The K⁺ channels activated by raising intracellular Ca²⁺ have been widely reported for various cells^{11–13}. The ATP-sensitive K⁺ channel in the present study, however, was observed in the absence of Ca²⁺ at the inner side of the membrane. Furthermore the activity of the channel was depressed by increasing the Ca²⁺ concentration to 10 μ M in the absence or presence of 0.5 mM ATP, indicating that the channel is different from the Ca²⁺-

mediated K⁺ channel. The ATP-sensitive K⁺ channel may also be different from the cation channel linked to the ATP receptors in mammalian sensory neurones (purinergic receptor; ref. 14).

The intracellular ATP concentration in normal cardiac cells has been reported to be 3–4 mM¹⁵. According to the present results, this value is high enough to block the ATP-sensitive K⁺ channel. In fact no significant outward current is activated in the plateau potential range in normal mammalian ventricular cells. The plateau of the cardiac action potential is shortened in hypoxic conditions^{1–4}, and the ATP content falls below 10% of the control, which is low enough to activate the ATP-sensitive K⁺ channels¹⁶. Thus, the marked increase in the outward current, which was reversed by an intracellular injection of ATP⁵, can be attributed to the activation of the ATP-sensitive K⁺ channel. The shortening of the action potential is accompanied by a decrease in contraction¹⁷ which is a major mechanism consuming ATP. Therefore, activation of the ATP-sensitive channel may prevent further depletion of ATP and protect the cell from irreversible impairment of its energy metabolism¹⁶.

More recently, Trube and Hescheler¹⁸ reported a similar ATP-sensitive channel in guinea pig ventricular cells.

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Hyperpolarization following activation of K⁺ channels by excitatory postsynaptic potentials

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We have postulated that an excitatory postsynaptic potential (e.p.s.p.) may open voltage-sensitive K⁺ ('M') channels¹, in an appropriate depolarizing range, and that this could alter the e.p.s.p. waveform. Consequently, the fast e.p.s.p. in neurones of sympathetic ganglia, elicited by a nicotinic action of acetylcholine (ACh)², could be followed by a hyperpolarization, produced by the opening of M channels during the depolarizing e.p.s.p. and their subsequent slow closure (time constant ~150 ms)¹. This introduces the concept that transmitter-induced p.s.ps may trigger voltage-sensitive conductances other than those initiating action potentials, and that in the present case this could produce a true post-e.p.s.p. hyperpolarization. (Some hyperpolarizations other than inhibitory postsynaptic potentials (i.p.s.ps) have been reported to follow e.p.s.ps^{3,4}.) We show here that this is so.

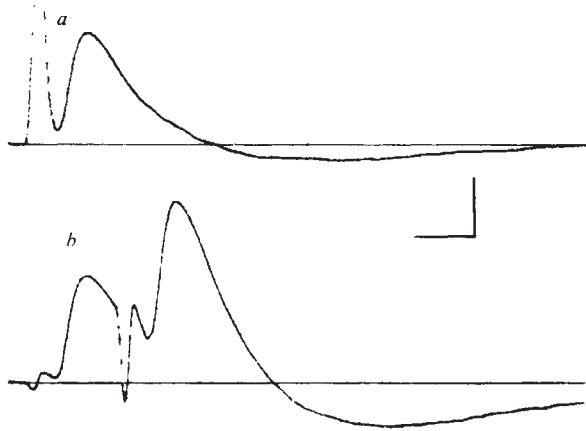


Fig. 1 Post-e.p.s.p. hyperpolarization and e.p.s.p. amplitude. Intracellular recordings are of a B neurone, partially curarized, with an average of 20 responses (Nihon Kohden KK) for each tracing. The bathing medium, held at 23–25 °C, contained 112 mM NaCl, 2 mM KCl, 1.8 mM CaCl_2 , 2.4 mM NaHCO_3 , 0.5 mM MgCl_2 and 5 mM glucose. Traces show the response to single stimulus (a) and to two preganglionic stimuli, the second of which elicited an e.p.s.p. with larger peak amplitude (b). Resting $V_m = -53$ mV for both a and b. The e.p.s.p. is preceded by a stimulus artefact and followed by the longer, tail-like post-e.p.s.p. hyperpolarization (downwards). (Differences in stimulus artefacts even for identical stimulus pulses are due to characteristics of the digital amplifier in the averaging device.) Calibration: 1.5 mV (intracellular negative, downwards) and 20 ms. For all intracellular studies of e.p.s.ps, orthodromic firing was routinely eliminated by moderately depressing the e.p.s.p. with (+)tubocurarine at 20–30 $\mu\text{g ml}^{-1}$ (30–44 μM). (However, post-e.p.s.p. hyperpolarization was also observed in the absence of (+)tubocurarine; such an e.p.s.p., free of an action potential, was produced when its orthodromic volley was timed to arrive during the refractory period following a preceding somatic action potential, elicited by a direct depolarizing stimulus via the intracellular electrode.) Atropine (1.4 μM) was routinely added to eliminate any hyperpolarizing slow i.p.s.p. component⁵, except when testing the effects of muscarine (as in Fig. 3a–c).

This proposal was studied in the 9th or 10th ganglion in the isolated sympathetic chain of the bullfrog (*Rana catesbeiana*) both with intracellular electrodes (3 M KCl) and with extracellular ones (surface Ag–AgCl wires in an air-gap chamber), as described previously^{3,5}.

At a given average membrane potential (V_m), close to -50 mV, following impalement, each e.p.s.p. exhibited an afterhyperpolarization whose amplitude increased (though not linearly) with increase in the amplitude of the e.p.s.p. (Fig. 1). However, the afterhyperpolarizations and the recovery phase of the e.p.s.ps were both voltage-dependent, whether changes in initial resting V_m were spontaneously encountered or imposed by applied current. With spontaneous resting V_m s between -45 and -60 mV, 54 cells all exhibited post-e.p.s.p. hyperpolarization, while five cells at potentials more negative than -70 mV showed none. When the resting V_m was altered by an applied current, the amplitude and duration of the post-e.p.s.p. hyperpolarization decreased progressively as V_m became hyperpolarized; the post-e.p.s.p. hyperpolarization disappeared at V_m values between -60 and -65 mV and did not reverse at more negative potentials (see Fig. 2). Conversely, during the recovery phase of the e.p.s.p. itself, there was less overlap by the hyperpolarizing component, so that the total duration of the e.p.s.p. increased to about 150 ms at V_m s between -65 and -70 mV (Fig. 2). The differences among reported time constants for the decay of e.p.s.ps (see refs 3, 6) may be explained by this influence of the resting V_m on post-e.p.s.p. hyperpolarization and the onset of the hyperpolarization process during the e.p.s.p.

The range of V_m in which post-e.p.s.p. hyperpolarizations can appear parallels closely that found for the opening of 'M' (K^+) channels¹. The post-action-potential hyperpolarizations

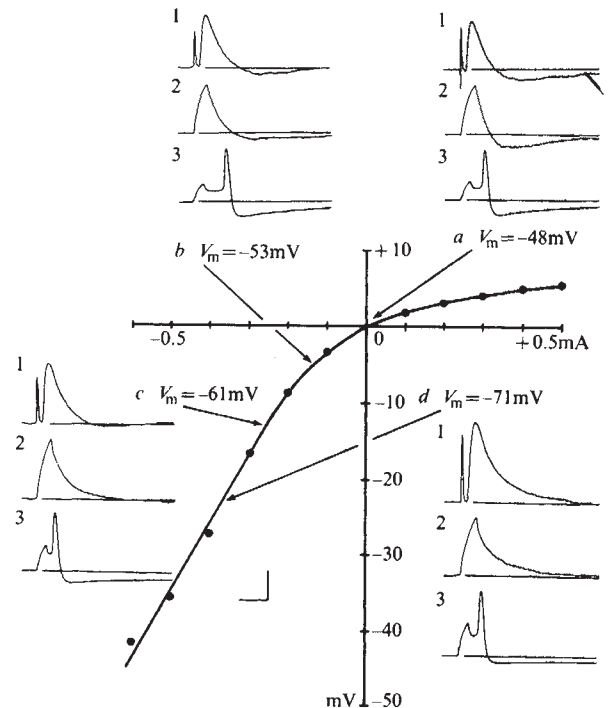


Fig. 2 Comparison of the voltage-sensitivities of the afterhyperpolarizations that follow: 1, e.p.s.ps; 2, applied depolarizing pulses; and 3, action potentials. A set of these three responses, in vertically descending order, is shown for each of four initial levels of V_m on the voltage-current (V – I) curve; a, initial $V_m = -48$ mV; b, -53 mV; c, -61 mV; d, -71 mV, with an arrow from each V_m indicating its position on the V – I curve. All responses shown were obtained with intracellular electrode in the same B neurone. Applied current was passed through the recording electrode, using a balancing bridge circuit to eliminate effects introduced by the electrode resistance. Points on the V – I curve represent steady-state values during 500-ms constant-current pulses, starting from resting $V_m = -48$ mV. In each group of three responses, the top tracing is the e.p.s.p. response, preceded by the single preganglionic stimulus artefact (1); the centre trace is the electrotonic potential induced by an applied constant outward current pulse, 0.09 nA for 20 ms (2); the bottom trace is the action potential fired by a direct intracellular stimulus (3 ms, outward current pulse, whose large artefact precedes each spike) (3). The top and middle tracings each represent an average of 20 responses, elicited at 1 per 5 s. Calibration: 2 mV and 40 ms for top and middle tracings; 40 mV and 10 ms for bottom tracings in each group of three responses. Note that after hyperpolarizations for both the e.p.s.ps and the electrotonic depolarizing pulses show a similar voltage-sensitivity; they disappear when the initial V_m is close to -60 mV and do not reverse at more negative V_m s. For the action potential, the afterhyperpolarization is still definite at $V_m = -71$ mV, and it could be reversed at V_m s negative to -85 or -90 mV.

also decrease with hyperpolarization, but they do not disappear at V_m s more negative than -60 mV (see Fig. 2) and they reverse at V_m s negative to E_K (about -85 to -90 mV)⁶. From our postulated explanation of post-e.p.s.p. hyperpolarization, as well as from the observed steady outward M current at V_m s depolarized by an applied voltage-step¹, it follows that an electrotonic depolarization, similar in form to an e.p.s.p. but induced by an applied current pulse rather than by the transmitter (ACh), should also be associated with an afterhyperpolarization having characteristics of post-e.p.s.p. hyperpolarizations. Such a result is seen in Figs 2 and 3.

If the post-e.p.s.p. hyperpolarization results from a temporary opening of M channels, in a suitable range of V_m the afterhyperpolarization should be selectively eliminated by muscarine, which closes the M channels¹, but not by tetraethylammonium (TEA), because it blocks the conventional 'delayed rectification' K^+ channels but not the M channels¹. Both of these predictions were borne out experimentally (Fig. 3).

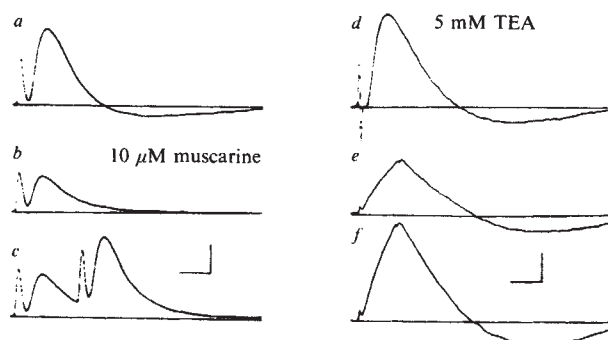


Fig. 3 Effects of muscarine and TEA on afterhyperpolarizations. *a-c*, Intracellular responses to orthodromic preganglionic input, resting V_m maintained at -57 mV; *a*, before, *b* and *c*, after addition of muscarine ($10 \mu\text{M}$). Muscarine eliminated the post-e.p.s.p. hyperpolarization, even when the e.p.s.p. amplitude was restored to pre-muscarine value by repetition of input in *c*. (The smaller amplitude of e.p.s.ps in muscarine is presumably due to an additional effect of muscarinic agonists in frog ganglia, namely a presynaptic depression of the release of ACh⁷.) *d-f*, TEA (5 mM) already present, and resting V_m maintained at -58 mV; afterhyperpolarizations still follow both the e.p.s.p. in *d* and the applied depolarizing pulses in *e*, *f* (constant current, 20 ms at 0.07 nA for *e* and 0.11 nA for *f*). That TEA was having its expected effect on other K⁺ channels was evident in a prolongation of the action potential (not shown). Calibration: 4 mV and 20 ms for *a-c*; 1 mV and 20 ms for *d-f*.

The above evidence supports the proposal that the post-e.p.s.p. hyperpolarization is a voltage-activated response; it is induced when the e.p.s.p. sufficiently depolarizes the membrane, and it can thus 'contaminate' the e.p.s.p. response. The dependence of post-e.p.s.p. hyperpolarization on the amplitude of the e.p.s.p. further indicates that it is a true afterhyperpolarization rather than a separate, transmitter-induced p.s.p.; the relationship was seen whether the amplitude of the e.p.s.p. was varied by repetition of input (as in Fig. 1) or by strength of curarization. (When e.p.s.ps were more strongly suppressed by greater curarization, post-e.p.s.p. hyperpolarizations were also smaller or absent.) That the voltage-activated post-e.p.s.p. hyperpolarization is a function of the opening of M channels by the e.p.s.p. is indicated by (1) the similarity of the range of V_m in which both appear, and (2) blockade of both by muscarine, but not by TEA. The more than linear increase in post-e.p.s.p. hyperpolarization when the amplitude of the e.p.s.p. was increased (Fig. 1) would be expected, as M channel conductances do show progressively greater increases with increasing depolarization of V_m in the range between -60 mV and -40 mV (ref. 1). Post-e.p.s.p. hyperpolarizations are of briefer duration and have smaller time constants than do those obtained for M-channel closure when a depolarized V_m is changed suddenly to a hyperpolarized holding level¹, but this is also readily explainable: M channels that are increasingly opened as e.p.s.p. depolarization rises, would begin to close progressively during the slow, descending, repolarizing limb of the e.p.s.p. Thus, the duration and time constant solely of the post-e.p.s.p. hyperpolarization phase that survives after the end of e.p.s.p. depolarization would not reflect the total time of the whole process.

As post-e.p.s.p. hyperpolarization in fact appears only in a limited range of V_m , its presence or absence can be used to indicate limits for values of the resting V_m of uninjured neurones, without directly measuring V_m using an intracellular microelectrode.

In 20 preparations, the extracellular (total population) e.p.s.p. response of intact frog ganglia⁵ was never followed by a post-e.p.s.p. hyperpolarization, whether elicited by a single stimulus or a train of maximal B or B plus C preganglionic volleys, when the true slow i.p.s.p. had previously been eliminated by atropine. On the other hand, during application of limited depolarizing current, passed via the surface recording

electrodes, the extracellular e.p.s.p. did exhibit a post-e.p.s.p. hyperpolarization in 5 of 11 intact ganglia tested. We conclude that the resting V_m of these intact sympathetic neurones is at least equal or negative to -65 mV. For neurones impaled by a microelectrode, mean resting V_m s of -50 mV (ref. 3), -54 mV (ref. 2) and -65 (range -55 to -75)⁶ mV have been reported.

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Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis

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Viruses have been found to induce inflammatory demyelinating lesions in central nervous system (CNS) tissue of both animal and man, either by natural infections or after vaccination^{1,2}. At least two different pathogenic mechanisms have been proposed for these changes, a cytopathic viral infection of oligodendroglia cells with subsequent cell death, and a host immune reaction against virus and brain antigens. We now report the occurrence of cell-mediated immune reactions against basic myelin proteins in the course of coronavirus infections in Lewis rats. Infection of rats with the murine coronavirus JHM leads to demyelinating encephalomyelitis developing several weeks to months post-infection³⁻⁷. Lymphocytes from these diseased Lewis rats can be restimulated with basic myelin protein (BMP) and adoptive transfer of these cells leads to lesions resembling those of experimental allergic encephalomyelitis (EAE) in recipients, which can be accompanied by a mild clinical disease. This model demonstrates that a virus infection in CNS tissue is capable of initiating an autoimmune response which may be of pathogenic importance.

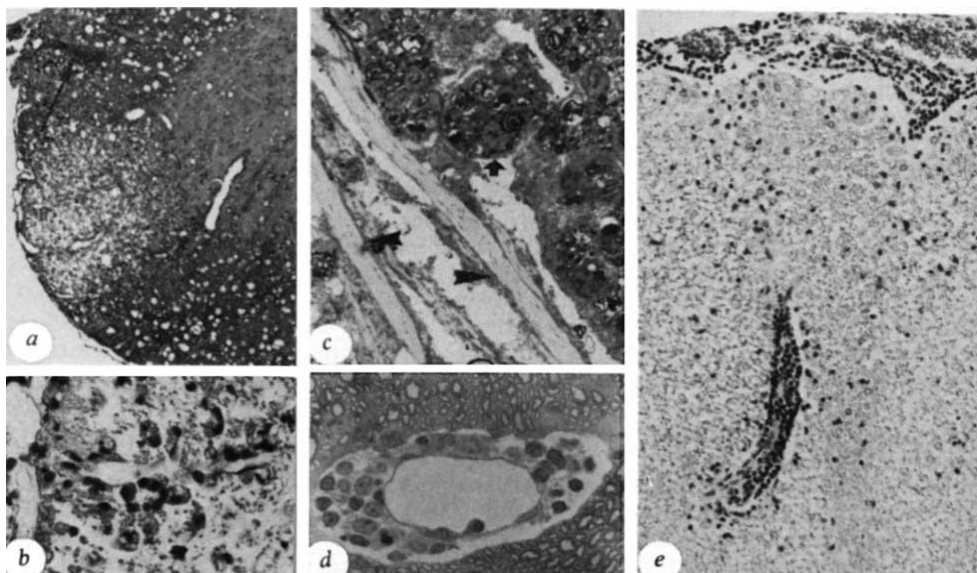
Intracerebral inoculation of Lewis rats at the age of 4-5 weeks with wild-type coronavirus JHM (1×10^4 plaque-forming units (PFU) per rat) was followed after an incubation period of several weeks by the development of a subacute demyelinating encephalomyelitis (SDE). Diseased animals revealed unsteadiness, ataxic gait, abnormal posturing of the limbs, hindleg paralysis or a complete paraplegia. Most of these animals recovered from SDE and only some rats died within a few days after onset of disease. The neuropathological changes consisted of demyelinated plaques which were distributed in thalamus, brain stem, cerebellum and spinal cord and were located predominantly in the white matter. Figure 1a shows such a plaque. Within the plaques, cell infiltrations which consisted mainly of macrophages (Fig. 1b, c), were found. As documented by Fig. 1c, axons were well preserved within the demyelinated area. Furthermore, perivascular cuffings were frequently observed in diseased rats (Fig. 1a). These cuffings were mainly found in the brain stem and spinal cord and were restricted to the space around the blood vessel (Virchow-Robins space, Fig. 1d). The destruction of myelin around these infiltrations was usually not prominent. Conventional virus isolation procedures permitted isolation of infectious coronavirus

Table 1 Proliferative response of lymphocytes cultured in presence of antigens

Group of rats	No. of animal	Control medium (c.p.m.)	BMP		Antigens JHM virus		Histone	
			(c.p.m.)	SI	(c.p.m.)	SI	(c.p.m.)	SI
SDE	1	2,454±685	20,477±933	×8.3	38,039±1,746	×15.5	2,944±139	×1.2
	2	5,913±539	39,815±4,118	×6.7	45,521±8,477	×7.7	5,321±482	×0.9
	3	1,638±84	4,366±202	×2.7	4,738±602	×2.8	1,801±203	×1.1
	4	3,866±295	4,530±666	×1.2	14,191±968	×3.7	3,692±464	×1.0
	5	2,650±96	6,244±680	×2.4	9,045±767	×3.4	2,411±284	×0.9
Average		3,304±1,486	15,086±13,748	×4.3±2.7	22,306±16,351	×6.6±4.8	3,233±1,214	×1.0±0.1
Uninfected	1	3,148±156	3,396±123	×1.1	2,833±301	×0.9	2,575±74	×0.8
	2	2,187±126	1,899±510	×0.9	2,361±174	×1.1	1,740±528	×0.8
	3	2,480±84	2,340±360	×0.9	1,984±223	×0.8	2,532±776	×1.0
	4	4,452±267	3,481±215	×0.8	4,814±215	×1.1	2,671±253	×0.6
	5	2,156±80	1,841±55	×0.9	2,324±118	×1.1	1,940±147	×0.9
Average		2,884±861	2,591±713	×0.9±0.1	2,863±1,012	×1.0±0.1	2,291±376	×0.8±0.1
EAE	1	2,086±148	5,038±748	×2.4	2,470±112	×1.2	ND	
	2	3,461±34	12,327±1,042	×3.6	1,409±65	×0.4	ND	
	3	2,532±260	5,214±86	×2.1	2,310±922	×0.9	2,713±765	×1.1
	4	2,679±61	9,405±284	×3.5	2,961±46	×1.1	ND	
Average		2,689±496	7,997±3,050	×2.9±0.6	2,287±561	×0.9±0.3		

Values summarize the counts per min (c.p.m.) and the stimulation indices (SI) obtained from five SDE rats, five uninfected animals and four rats with EAE. Lymphoid cells from SDE rats were taken 2 days after onset of disease (2–3 weeks after infection). EAE animals were killed 2 weeks after BMP immunization. Immunization was carried out according to Richert *et al.*⁸. From individual animals cells from spleen, thymus and peripheral blood were pooled. As lymphocytes from popliteal lymph nodes could only be obtained from EAE animals, this cell population was not included in the comparative study. Cells were cultured in 96-well microplates with RPMI 1640 supplemented by heat-inactivated fetal calf serum, 2-mercaptoethanol, antibiotics and sodium carbonate, at a cell density of 1×10^6 per well for 72 h, with the addition of feeder cells (1×10^5 mitomycin C-treated spleen cells). Before incubation, 1 µg per well of the required antigen was added. 1 µCi ^3H -thymidine was added to each well 24 h before collection and the stimulation indices were calculated and compared with those of cultures without antigen⁸. As a source of BMP, guinea pig spinal cords were extracted according to the method described previously¹⁸ to obtain a potent antigen for the specific restimulation of sensitized lymphocytes. To exclude the presence of JHM virus in lymphocytes from SDE animals, lymphoid cell aliquots of the cell preparations used for stimulation experiments were processed for virus isolation before and after stimulation with BMP or concanavalin A. Cells were distributed on monolayers of L cells in Costar plates, or homogenized before adsorption to L cells and incubated at 37 °C for 3 days. Infectious virus was not isolatable even after two blind passages. In addition, lymphoid cells from healthy rats (2 months old) were exposed to JHM virus at different multiplicities for 1 h at 37 °C and further cultured after washing. Again, no infection of lymphocytes was noted and no JHM antigen was detectable in either of the cell preparations, suggesting that rat lymphoid cells do not support replication of JHM virus. ND, not done.

Fig. 1 *a–d*, Lewis rat with onset of SDE 20 days post-infection. *a*, Demyelinated plaques in cervical spinal cord in antero-lateral white matter. Note perivascular cell infiltration (arrow). Double-staining with haematoxylin–eosin and Luxol fast blue. ×51. *b*, Higher magnification (×248) of the same plaque. Myelin is almost completely absent and infiltrated macrophages contain myelin debris. *c*, 1-µm section from same level as *a* and *b*. Well-preserved demyelinated axons (arrowheads) are seen. Macrophages contain myelin debris (arrow). Toluidine blue stain. ×443. *d*, Perivascular cell infiltration in the spinal cord of the same rat. Toluidine blue staining. ×359. *e*, Transferred perivascular EAE-like lesions in the posterior column and meninx of the spinal cord. The animal shown in *a–d* was perfused with glutaraldehyde–paraformaldehyde after lymphocytes had been taken for stimulation assays. The tissue specimens were embedded in paraffin (*a, b*) or in epon after fixation in osmium tetroxide (*c, d*) for 1-µm sections. The rat shown in *e* received 1×10^8 lymphocytes from an animal with SDE after *in vitro* culture with BMP. Paraffin-embedded section, haematoxylin–eosin staining. ×125.



from brain or spinal cord at the time of clinical disease and up to 10–12 days after inoculation. Viral antigen was primarily detectable by immunohistological procedures in glia cells in the neighbourhood of demyelinating plaques and not in cells constituting perivascular cuffs.

The marked infiltration of lymphocytes in the affected brain areas (Fig. 1*d*) to some extent resembles changes seen in EAE.

This observation prompted us to perform immunological studies to evaluate the state of lymphocyte sensitization against BMP. Lymphocytes from individual Lewis rats with SDE were collected from spleen, thymus and peripheral blood 2 days after onset of disease and cultured for 3 days in the presence of BMP. As shown in Table 1, the lymphocytes of most rats were sensitized against BMP as measured by ^3H -thymidine

Table 2 Adoptive transfer of cultured lymphoid cells to rats

Cells transferred		EAE-like lesions in recipients		Detection of JHM virus in brain or spinal cord in recipients	
Donor	Antigen stimulation	Clinical	Histological	Infectious virus	Antigen
SDE rats	BMP for 3 days	6/26	13/26	0/12	0/12
	None for 3 days	0/5	0/5	0/3	0/3
Normal rats	BMP for 3 days	0/8	0/8	0/2	0/2
Normal rats	BMP for 3 days + JHM virus	0/5	0/5	0/5	0/5
Lymphoid cells from normal rats + JHM virus		0/4	0/4	0/4	0/4
Intravenous inoculation of infectious JHM virus into normal rats		0/6	0/6	0/60/6	

Spleen, thymus and peripheral blood cells from SDE rats were taken within 2 days after onset of clinical symptoms and pooled to obtain a maximum cell yield. Lymphoid cells at a density of 1×10^7 per ml were incubated with or without $10 \mu\text{g ml}^{-1}$ BMP extracted from guinea pig spinal cord. After 72 h the cells were collected and washed four times in Hank's balanced salt solution. 1×10^8 viable cells were transferred i.v. to normal syngeneic rats. Recipients without clinical signs were killed on day 10 after cell transfer⁸. For virus isolation attempts the left brain hemisphere and cervical part of the spinal cord from control rats were homogenized and inoculated on monolayers of L cells on Costar plates according to the method described previously⁷. Part of the right brain hemisphere and the spinal cord were processed for immunohistology with the peroxidase-antiperoxidase method. As additional control experiments, BMP-restimulated and unstimulated lymphoid cells from normal rats (2 months old) were exposed to JHM virus at multiplicities of 0.1–1, and transferred i.v. to rats. Each rat received $1-3 \times 10^8$ viable cells. Also, JHM virus (10^6-10^8 PFU per animal) was injected i.v. into rats (2 months of age). Both experiments were terminated 10 days after cell transfer or virus inoculation.

incorporation. The stimulation indices observed were similar to values for lymphocytes from rats with EAE and greater than the values obtained using cells from uninfected animals. Lymphocytes from SDE animals were also easily restimulated by purified inactivated coronavirus particles. Interestingly, neither infectious JHM virus nor JHM antigens were detectable in lymphoid cells from SDE rats before or after stimulation with either specific antigen or mitogen.

To discover whether lymphocytes from rats with SDE could induce histopathological changes in the CNS, cells restimulated with BMP were transferred into healthy recipients by the intravenous (i.v.) route⁸. The results of these experiments are summarized in Table 2. After transfer of 1×10^8 lymphocytes per animal, 6 of 26 animals showed slight clinical signs such as weight loss, abnormal sensitivity to touch or slight ataxic gait. These clinical signs were seen within 5 days of transfer. Perivascular mononuclear cell infiltration was observed histologically in 13 animals. An example of such an infiltration is shown in Fig. 1e. This type of lesion was similar to EAE, or passively transferred EAE⁸. The infiltrations were predominantly found in the dorsal area of the spinal cord white matter. This location of lesions could explain the low frequency of clinical symptoms. Additional perivascular cuffs were detected in the pons, cerebellar white matter and thalamus. Small scattered cell infiltrations were visible near the perivascular cuffs but no extended demyelination was detectable. No lesions were observed if lymphocytes from uninfected rats were transferred after culture in presence of BMP, or after exposure to infectious JHM virus. It is unlikely that these lesions are caused by transfer of coronavirus because we were unable either to reisolate infectious virus from lymphocytes from rats with SDE, or to detect infectious JHM virus or antigens in brain areas with lesions from animals which received restimulated lymphocytes. Furthermore, i.v. inoculation of normal rats with high doses of virus or virus-lymphocyte mixtures did not lead to CNS lesions.

The destruction of oligodendroglia cells by viruses has been shown to have a role in the development of demyelination in experimental models. However, only circumstantial evidence exists that a virus infection initiates an immune response which contributes to brain injury as suggested in infections with canine distemper, Theiler, visna or vaccinia viruses⁹⁻¹⁴. The data presented here indicate that in the course of coronavirus JHM infection of Lewis rats, lymphocytes were sensitized against BMP. Adoptive transfer of these lymphocytes after *in vitro* restimulation with BMP was followed by EAE-like lesions in recipient Lewis rats. Coronavirus replication in the CNS can therefore produce a similar effect to Freund's complete

adjuvant plus BMP in EAE. It will be of great importance to understand how a virus infection triggers an immune response against neuroantigens. There are several possibilities. First, it is conceivable that JHM virus structural proteins which are formed in CNS cells contain antigenic sites which cross-react with myelin. An immune response against these proteins would therefore be directed against both virus proteins and myelin. Some evidence for such a mechanism has been obtained for measles virus infection in subacute sclerosing panencephalitis, in which antibodies to measles virus reacted with BMP¹⁵. However, cell-mediated immune reactions were not measured. Second, the infection of oligodendroglia cells by JHM virus may lead to membrane changes resulting in the development of immunogenic structures also recognized on uninfected cells thus inducing an autoimmune process. So far, this mechanism has been suggested for viruses which bud from cell membranes¹⁶. How coronaviruses are released from infected cells is unknown, but it is possible to detect viral antigens on the cell membrane. The role of these membrane-associated viral antigens in assembly or release is unknown. Third, coronavirus infection of oligodendroglia cells could lead to the release of myelin material which is immunogenic. The resulting series of events would then follow those of EAE. There has been some evidence that a persistent measles virus infection in hamsters supports the development of EAE when such animals are challenged with BMP in combination with Freund's complete adjuvants¹⁷. Whether the measles infection renders the CNS more vulnerable to immunological injury or immunopotentiates a cell-mediated immune response to myelin has not yet been elucidated.

None of these different mechanisms has been proven for any of the various models established for virus-induced demyelination, but coronavirus JHM infection in Lewis rats may now permit such an analysis. In addition, this model may provide significant information in the study of chronic inflammatory CNS diseases of man such as multiple sclerosis.

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Defective translation of measles virus matrix protein in a subacute sclerosing panencephalitis cell line

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Subacute sclerosing panencephalitis (SSPE) is a slowly progressing fatal human disease of the central nervous system (CNS) that is associated with measles virus persistence. Virus nucleocapsids are present in the brain^{1,2} and the patient is in a state of hyperimmunization towards this agent. However, although all other structural polypeptides are recognized by the immune system, there is a markedly decreased antibody response towards virus matrix or membrane protein^{3,4}. Matrix protein has not been detected in brain cells⁵ and infectious virus is not present. The absence of this virus structural polypeptide is thought to account for the apparent restriction in virus maturation both *in vivo* and *in vitro*. SSPE viruses can only rarely be rescued from brain tissue by co-cultivation or cell fusion techniques using tissue culture cell lines susceptible to measles virus infection⁶. Often this procedure fails to yield a lytic budding virus but produces instead a carrier cell line in which the agent is cell associated. These lines (known as SSPE cell lines) also do not contain matrix protein^{7,8}. However, the reason for this deficiency is unknown. We have therefore now examined an SSPE cell line which does not yield infectious virus in order to define this process further. We found that although messenger RNA for membrane protein was present, it was unable to form normal matrix protein in translation reactions.

In our laboratory we have examined an SSPE cell line (N-1) obtained by co-cultivation of SSPE brain material with Vero cells⁹. This cell line shows slowly spreading areas of cell fusion, but does not release infectious virus, although particles can be obtained by procedures which favour cell fragmentation^{10,11}. During ordinary cell culture procedures, or in co-cultivation and cell fusion experiments, we have never detected release of infectious virus. Results of electron microscopic examination of the N-1 cells are consistent with a defect in virus maturation, and the virus nucleocapsids are randomly distributed throughout the cytoplasm¹². These cells are thus a good model for the situation observed in CNS cells from SSPE patients.

Measles virus proteins can be detected in infected cells by immunoprecipitation and comprise: the large protein (L), haemagglutinin (H), phosphoprotein (P), nucleocapsid (N) and its degradation product (NC), the fusion protein, both uncleaved (F₀) and cleaved (F₁), and the matrix protein (M). The P protein is poorly recognized by the serum used in this work, but the small band (X) detected in infected cells and known to be related¹³ to P was precipitated from both cell lysates and *in vitro* translation products. We confirmed that this protein is unrelated to matrix protein using the technique of Cleveland mapping²⁵. All of these proteins were detected in lysates of

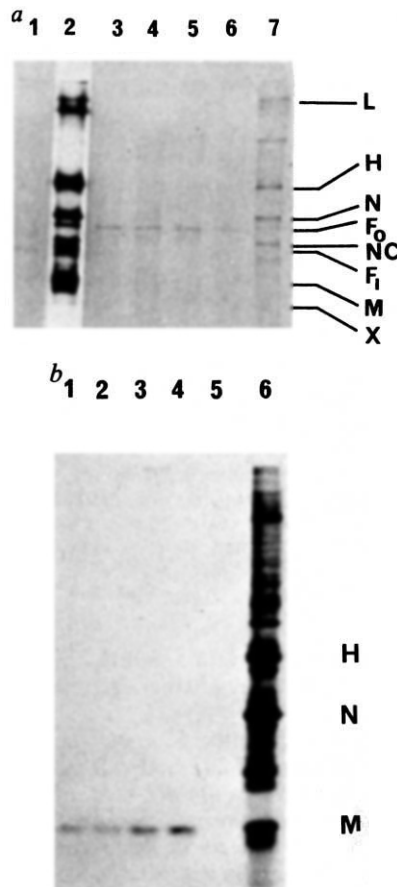


Fig. 1 *a*, Immunoprecipitation of measles-specific proteins from N-1 cells. Cells were labelled with ³⁵S-methionine (1,200–1,400 Ci m^{mol}⁻¹, Amersham Buchler) for 2 h when the cytopathic effect was maximal. Lysates were prepared and analysed by immunoprecipitation and electrophoresis on a 10% SDS-polyacrylamide gel as described elsewhere³. Gels were fluorographed and exposed to Fuji X-ray film. Samples were precipitated with: tracks 1, 2, 7, rabbit hyperimmune anti-measles serum; track 3, control ascites fluid; tracks 4–6, ascites fluids containing monoclonal antibodies directed against Edmonston measles virus matrix protein. Track 1, lysate prepared from uninfected Vero cells; track 2, lysate prepared from Edmonston virus-infected Vero cells; tracks 3–7, lysates prepared from N-1 cells. *b*, Specificity of monoclonal antibodies. Lysates were prepared from Edmonston measles virus-infected Vero cells and immunoprecipitated with: tracks 1–4, anti-measles virus matrix protein monoclonal antibodies; track 5, monoclonal antibody raised against coronavirus; track 6, total cell lysate, without immunoprecipitation.

Edmonston measles virus-infected Vero cells. All except matrix protein could be demonstrated in lysates prepared from N-1 cells (Fig. 1*a*). Matrix protein was also not detected by immunofluorescence or radioimmunoassay using four monoclonal M-specific antibodies (Fig. 1*b*) which also cross-react with the non-defective SSPE virus Lec¹⁴. However, the levels of virus protein expression were low in these cell populations, as only a small percentage of cells (10–20%) were infected. Analysis by radioimmunoprecipitation was complicated by background host-cell proteins (Fig. 1, track 1) and nucleocapsid degradation products which migrated in the matrix protein region (38,000 molecular weight). The long exposures required to search for trace amounts of matrix protein were found unsatisfactory, and it was therefore necessary to analyse protein production *in vitro*. This results in a decreased background in immunoprecipitation¹⁴. Matrix protein production could be prevented by a defect in either transcription or translation, and it is known that this inhibition is to some extent host-controlled, as cell fusion experiments have occasionally resulted in the rescue of infectious virus⁶. Therefore, *in vitro* translation was

Fig. 2 *In vitro* translation of mRNA from persistently infected cells. *a*, Track 1, sample immunoprecipitated with rabbit anti-measles antiserum, preadsorbed with uninfected Vero cell antigens; tracks 2–5, samples immunoprecipitated with different monoclonal antibodies specific for matrix protein. *b*, Protein products from the *in vitro* translation of mRNA isolated from N-1 SSPE cells (track 1) and uninfected (track 2) without immunoprecipitation. mRNA was extracted from cytoplasmic preparations of uninfected Vero, N-1 and carrier Lu106 cells²² and translated in a rabbit reticulocyte lysate system²³ (provided by Dr S. Siddell). Products were diluted in RIPA buffer containing protease inhibitors³ and immunoprecipitated using rabbit anti-measles virus serum preadsorbed with uninfected Vero cell antigens. The same products were also immunoprecipitated with four monoclonal antibodies directed against Edmonston virus matrix protein. Samples were then analysed on a 10% SDS-polyacrylamide gel and visualized by fluorography. Positions of ¹⁴C-labelled molecular weight markers (Amersham) are indicated.

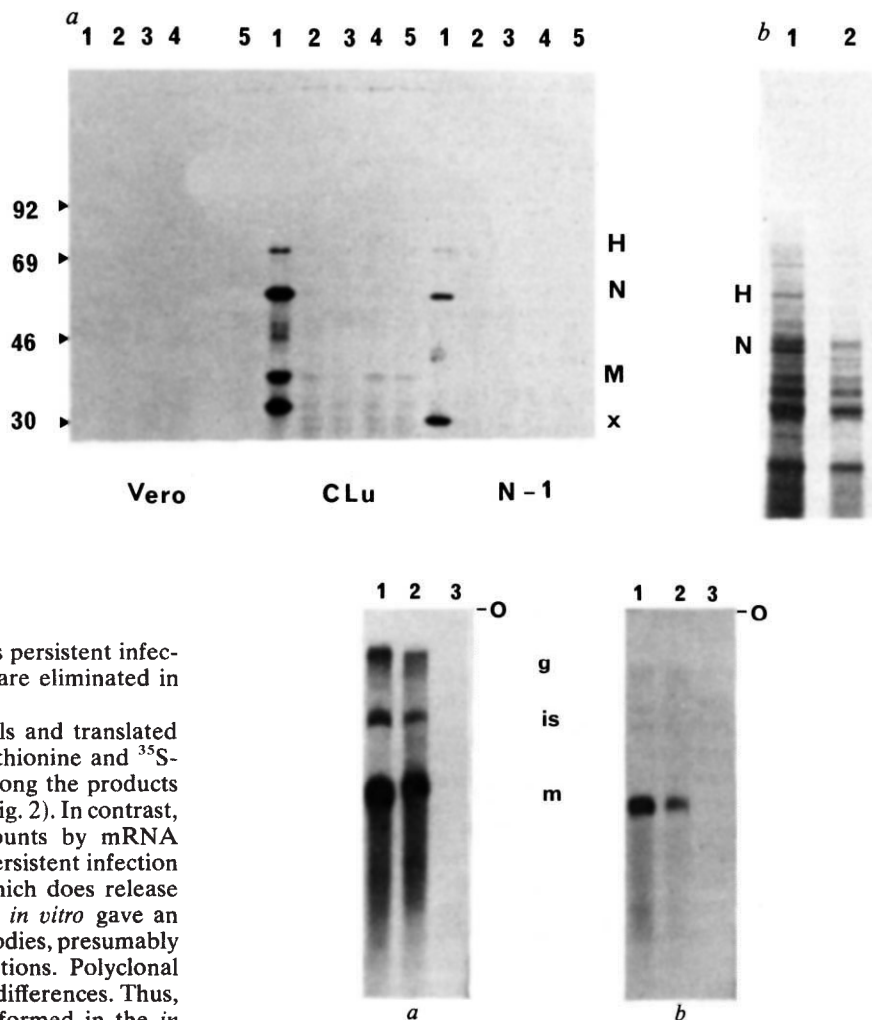


Fig. 3 Analysis of mRNA by filter hybridization. The mRNA preparations translated in Fig. 2 (1 µg per track) were denatured in formamide and separated at 100 mA on a 1.5% agarose gel buffered with MOPS and containing 5% formaldehyde (37%, Merck). RNAs were then transferred in 1 × SSC to nitrocellulose filters (NEN Gene-Screen)²⁴. Filters were baked at 80 °C for 2 h and hybridized to ³²P-labelled plasmids (pBr322, 6–9 × 10⁸ c.p.m. per µg) containing inserted sequences derived from Edmonston measles virus nucleocapsid (panel *a*) and matrix (panel *b*) mRNAs. In each panel: track 1, mRNA from carrier Lu106 cells; track 2, mRNA from SSPE-N-1 cells; track 3, mRNA extracted from uninfected Vero cells. O, Origin; g, genome; is, intermediate sized RNA; m, mRNA.

used to investigate such control in SSPE virus persistent infections¹⁵; putative host-cell regulatory factors are eliminated in these experiments.

When mRNA was extracted from N-1 cells and translated in an *in vitro* system containing both ³⁵S-methionine and ³⁵S-cysteine, matrix protein was not detected among the products either with or without immune precipitation (Fig. 2). In contrast, matrix protein was produced in large amounts by mRNA extracted from carrier Lu106 (CLu) cells, a persistent infection initiated with measles virus Edmonston¹⁶ which does release infectious particles. Matrix protein produced *in vitro* gave an unsatisfactory reaction with monoclonal antibodies, presumably because the protein lacked certain modifications. Polyclonal rabbit antiserum was not so sensitive to these differences. Thus, the conclusion that matrix protein was not formed in the *in vitro* translation reaction rests primarily on immunoprecipitation performed using this rabbit serum. As N-1 SSPE-cells did not form matrix protein *in vitro*, and as we were never able to demonstrate the protein by short pulse-labelling of tissue cultures, we were able to exclude completely the possibility that the protein is very unstable within the cell and rapidly degraded¹⁷.

The observed failure in matrix protein production could result from production of a defective mRNA, or from failure to produce that message at all. Accordingly, mRNAs isolated from CLu and N-1 cell lines, whose translation products are shown in Fig. 1, were analysed by electrophoretic separation on an agarose gel, transfer to nitrocellulose and hybridization to nick-translated, cloned cDNA copies of sequences contained in the measles virus Edmonston mRNAs for nucleocapsid and matrix proteins^{18,19}. The results of this analysis are shown in Fig. 3. In both cell lines examined, three classes of RNA were detected. The largest consists of the virus genome (g), and migrates with a true molecular weight of 4.5 × 10⁶ in a fully denaturing gel²⁰. The intermediate-sized RNA molecules (is) are of an unknown nature although they were observed to remain in the A(–) fraction after all mRNA had been removed. Such molecules might represent defective interfering RNAs, polymerase mistakes or RNA processing intermediates and may be similar to the polycistronic messages observed in vesicular stomatitis virus infections²¹. The smaller molecular weight species was the most plentiful molecule in the A(+) fraction and represented mRNA^{18,19}. A mRNA for nucleocapsid protein was readily demonstrated in both cell lines (Fig. 3*a*), providing an internal, positive control for these experiments.

When cloned copies of the matrix protein mRNA were used as a probe, mRNA was still demonstrable in both cell lines (Fig. 3*b*). The quantitative reduction in the level of matrix

protein mRNA in N-1 cells observed in Fig. 3*b* cannot, however, explain the lack of matrix protein. The amount of this mRNA in N-1 cell preparations often exceeds that in samples prepared from other infections in which matrix protein production is readily demonstrable (see Fig. 4).

Messenger RNA obtained from two normal Edmonston virus infections was analysed by the blotting technique. The amount of matrix protein mRNA in 1 µg of the N-1 cell sample was equivalent to that contained in 1 µg of the second Edmonston-infected cell sample (Fig. 4*a*, tracks 3, 6). When these mRNAs were translated *in vitro*, M protein was readily detected in the immunoprecipitated products of both Edmonston-infected cell mRNAs, but not in those specified by the N-1 cell message (Fig. 4*b*, track 6). Thus, we conclude that the N-1 SSPE cell line clearly contained a mRNA specific for matrix protein which could not be translated to produce the normal protein *in vitro* or in infected cells. The basis of this defect is unknown but it could arise through premature termination or failure to initiate translation. A small peptide or a protein which might be completely different antigenically from normal matrix protein (for

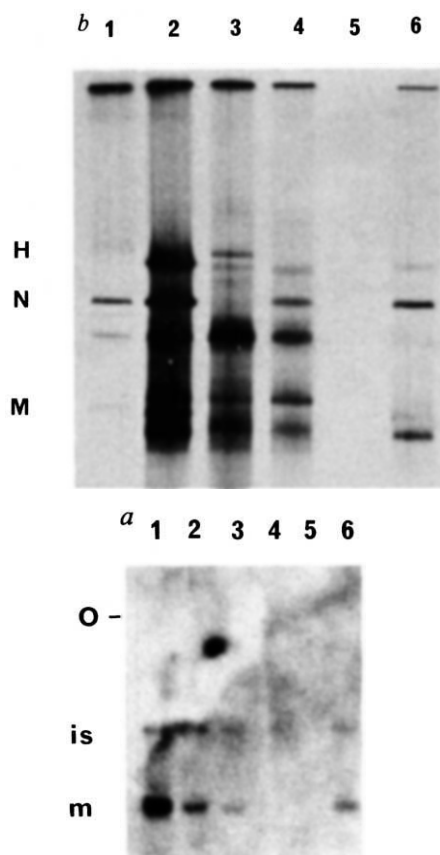


Fig. 4 *a*, Relative quantitation of mRNA samples. Tracks 1, 2, mRNA sample obtained from Edmonston virus-infected Vero cells (preparation 1); tracks 3, 4, mRNA sample from Edmonston-infected Vero cells (preparation 2); track 5, uninfected Vero cell mRNA; track 6, N-1 cell mRNA. 1 µg of mRNA was loaded onto tracks 1, 3, 5 and 6; 0.2 µg of mRNA were loaded onto tracks 2 and 4. *b*, *In vitro* translation. Track 1, Edmonston virus proteins immunoprecipitated from virus-infected Vero cell lysates proteins immunoprecipitated from translation products specified by; track 2, Edmonston virus-infected Vero cell mRNA (preparation 1); track 3, uninfected Vero cell mRNA; track 4, N-1 cell mRNA; track 5, No mRNA addition; track 6, Edmonston virus-infected Vero cell mRNA (preparation 2).

Methods: In *a*, two samples of mRNA isolated from measles virus (Edmonston)-infected Vero cells were electrophoresed with mRNA isolated from N-1 cells on a 1.5% agarose gel as described in Fig. 3 legend. The RNA was then transferred to a nitrocellulose filter and hybridized to ³²P-labelled DNA containing sequences derived from the matrix protein mRNA. In *b*, mRNA (1 µg) derived from uninfected cells, SSPE N-1 cells or Edmonston virus-infected Vero cell mRNA (preparations 1 and 2) were translated in the rabbit reticulocyte lysate system. The products from these reactions were immunoprecipitated using rabbit anti-Edmonston virus serum, analysed on a 10% SDS-polyacrylamide gel and visualized by fluorography. Antiserum used in this experiment was not preadsorbed with uninfected cell antigens and therefore recognized several host proteins contaminating the original virus antigen preparation (track 3).

example, due to a frameshift mutation) would not be detected in these experiments.

Whatever the molecular mechanism, the end result must be a lack of functional matrix protein in the infected cell. Matrix protein is thought to act as a trigger in the budding process, bringing together internal nucleocapsid structures and virus glycoproteins inserted in the cell membrane. Consequently, any defect in matrix protein production should lead to a cell-associated phenotype. The mRNA defect described here is only one process which could produce this effect. A Sendai virus persistent infection *in vitro* is known in which a drastic reduction in matrix protein stability within the cytoplasm leads to a similar deficiency in the levels of functional protein¹⁷. A transcriptional

defect could also accomplish the same end. It is therefore possible that measles viruses might adopt different strategies in order to achieve persistence. No single mechanism has yet been identified *in vivo*. However, this report constitutes the first such identification in an SSPE cell line *in vitro* and we are now searching for matrix protein-specific information in post-mortem samples of SSPE patient brain.

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Reversible induction of natural killer cell activity in cloned murine cytotoxic T lymphocytes

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Natural killer (NK) activity is a poorly understood component of the immune system, generally identified as the ability to kill certain tumour cells^{1,2}. Perhaps the most controversial issue has been the lineage to which cells displaying this activity belong. Extensive studies of surface antigens on cells with NK activity in both mouse and man have led to enigmatic results, such cells apparently bearing markers of both T-cell (Thy-1 and E receptor) and myeloid (Mac-1 and OKM1) lineages³⁻⁶. A fresh approach to this problem would be to take cells of known lineage and test whether they express, or could be induced to express, NK cell function. Using this approach we show here that monoclonal cytotoxic T lymphocyte (CTL) lines can be induced, by culture in high concentrations of spleen cell supernatant, to express a new lytic activity apparently identical with that of splenic cells NK activity. Preliminary evidence implicates both interleukin-2(IL-2) and interferon (IFN) as mediators of this phenomenon. These findings clearly demonstrate that cells of T cell lineage have the capacity to express NK activity.

Antigen-specific CTL clones were generated from single precursor cells using a standard protocol⁷. They were characterized in the following manner. (1) They had the surface marker profile of the CTL lineage, that is, Ig⁻, Thy-1⁺, Ly-2⁺. (2) Proliferation was greatly augmented by stimulation with spleen cells bearing

Table 1 Cytotoxicity of 2 CTL clones

Target cells	H-2	% Cytotoxicity AIG7	CIF2
CBA/J	k	2	5
C57BL/6	b	28	4
BALB/c	d	1	—
B6-C-H-2 ^{bml}	bml	—	66

The cytolytic specificity of AIG7 and CIF2 CTL clones on lipopolysaccharide (LPS) blast cells prepared from various strains of mice. Cytotoxicity was measured at a 1:1 effector:target cell ratio. AIG7 is a CBA/J anti-C57BL/6 (k anti-b) clone, and CIF2 is a C57BL/6ByJ anti-B6-C-H-2^{bml}/ByJ (b anti-bml) clone. **Methods:** CTL clones were generated essentially as described by Glasebrook and Fitch⁷. Briefly, spleen cells were stimulated with allogeneic irradiated (2,000 R) spleen cells through three successive weekly cycles of mixed lymphocyte culture. Cultures were set up in 16 mm diameter wells (Costar) in Click's medium containing 10% fetal bovine serum (FBS; Sterile Systems). Click's medium was prepared as described¹⁹, except HEPES buffer and antibiotics were omitted, and the sodium bicarbonate concentration was 0.22%. In the primary culture, 7×10^6 responder cells were mixed with 1.4×10^6 stimulator cells; in secondary and tertiary cultures, 2×10^6 and 5×10^5 responder cells, respectively, were mixed with 5×10^6 stimulator cells. At the end of each culture, viable cells were purified out by centrifugation on Ficoll-Hypaque (LSM Solution; Litton Bionetics). After 1–2 days of tertiary culture, the cells were resuspended in medium containing 2% rat spleen cell supernatant. This was prepared by culturing W/Fu spleen cells at 10^7 nucleated cells per ml in RPMI-1640 containing 5×10^{-5} M 2-mercaptoethanol, 5% FBS and 10 μ g/ml Con A (Pharmacia). Aliquots of 50 ml were cultured in 75 cm² flasks (Corning). Within 1–2 weeks the proliferating CTL were cloned at 1 cell per well into 6 mm diameter flat-bottomed wells (Falcon) containing 5×10^4 irradiated (2,000 R) thioglycollate-induced stimulator-strain peritoneal exudate cells. After 2 weeks, wells containing visible colonies were restimulated with irradiated spleen cells. Clones were grown routinely in 16 mm diameter wells in Click's: 10% FCS:2% rat spleen cell supernatant, and were stimulated at 1–2 week intervals with 7×10^6 irradiated stimulator-strain spleen cells, followed by recovery of viable cells on Ficoll-Hypaque 3–4 days later. LPS blasts were obtained by culturing spleen cells for 2 days in RPMI-1640 supplemented with 5×10^{-5} M 2-mercaptoethanol, 5% FBS, and 50 μ g ml⁻¹ LPS. For cytotoxicity assays, target cells were labelled with 100 μ Ci ⁵¹Cr-sodium chromate (NEN) for 2 h at 37°C. Washed target cells (5×10^3) were mixed with various numbers of effector cells in 200 μ l of RPMI-1640:5% FBS in V-bottomed microtest plates (Dynatech). Plates were spun at 200 g for 1 min and incubated at 37°C for 4 h. Aliquots of 100 μ l of supernatant were harvested and counted for radioactivity. Per cent cytotoxicity was calculated as $100 \times (\% \text{ release with effectors} - \% \text{ release in medium}) / (100 - \% \text{ release in medium})$.

the specific immunizing antigen, demonstrating that the responder clones bore antigen specific receptors. (3) The cloned lines showed high antigen-specific cytotoxicity. For example, the CBA/J anti-C57BL/6 clone, AIG7, lysed C57BL/6 blasts but not syngeneic CBA/J or third party BALB/c blasts, whereas the C57BL/6 anti-bml clone, CIF2, lysed bml blasts but not syngeneic C57BL/6 or third party CBA/J blasts (Table 1).

If these CTL clones were transferred from their normal growth medium, containing 2% concanavalin A (Con A)-induced spleen cell supernatant, to medium containing a 40% concentration of such supernatant, the cells began to proliferate rapidly and became markedly enlarged. When tested on blast cells, the specificity was unchanged, although a two- to threefold increase in lytic activity against the specific target sometimes was noted. By contrast, when tested on tumour target cells a dramatic alteration in specificity was observed (Fig. 1). For example, the CBA/J anti-C57BL/6 (k anti-b) clone AIG7, grown in medium containing 2% spleen supernatant, lysed only the H-2^b-bearing target, EL4. But after 1 week of growth in medium containing 40% supernatant, AIG7 cells lysed not only EL4, but also NK sensitive targets. Thus, both YAC-1 and an NK sensitive clone (27v-IC2) of the L5178Y lymphoma were readily lysed, whereas P815 and an NK resistant clone (av) of the L5178Y lymphoma were undamaged. In these respects, the

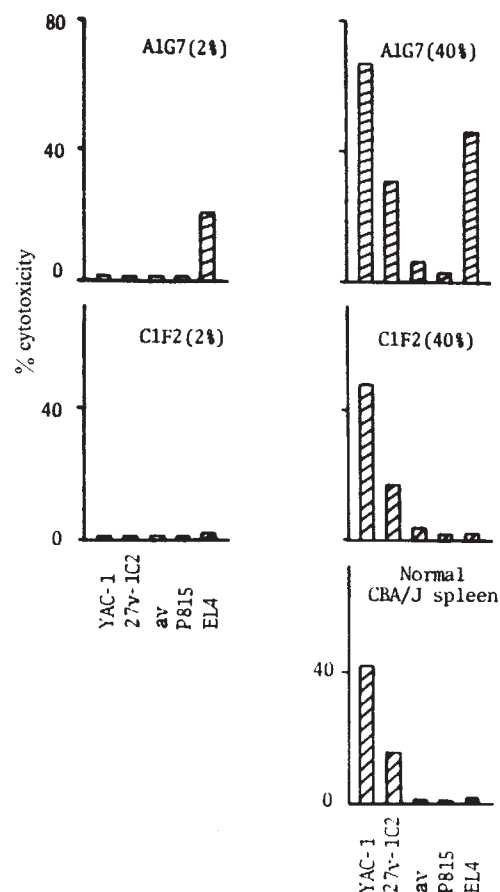


Fig. 1 Induction of NK activity in cloned CTL. Left hand side: specificity of AIG7 and CIF2 cultured in 2% rat Con A induced spleen cell supernatant. Right hand side: specificity of AIG7 and CIF2 after 1 week of culture in medium containing 40% mouse Con A induced spleen cell supernatant. CTL clones were placed in 40% supernatant 3 days after antigen stimulation. For comparison, the specificity of normal CBA/J spleen cells is shown at bottom right. The effector:target ratios were respectively 1:4, 1:1 and 100:1 for AIG7, CIF2 and normal spleen cells. The following target cells were used: YAC-1, an A/Sn (H-2^a) Moloney-virus induced lymphoma²⁰; the NK sensitive clone 27v-IC2 and NK-resistant clone 27av variants of the L5178Y methylcholanthrene-induced lymphoma of DBA/2 (H-2^d) mice²¹; P815, a methylcholanthrene-induced mastocytoma of DBA/2 mice²²; and EL4, a benzpyrene-induced leukemia of C57 (H-2^b) mice²³. YAC-1 and L5178Y tumours were maintained *in vitro* in RPMI-1640/10% FBS. P815 and EL4 were maintained as ascites in DBA/2 and C57BL/6 mice respectively. Mouse spleen cell supernatant was prepared from BALB/c mice in an identical manner to rat spleen cell supernatant (see legend to Fig. 1).

newly induced lytic activity was identical to that of splenic NK cells (see Fig. 1), and the monoclonal CTL line now showed both CTL and NK function. Culture in 40% spleen cell supernatant for 1 week caused a 3-fold increase in lytic units per 10^4 effector cells against the specific target, EL4, and a >100 fold increase in lytic units per 10^4 effector cells against YAC-1 targets. Similar results were obtained with a C57BL/6 anti-bml clone: control cells grown in 2% spleen cell supernatant lysed none of the five tumour cells in the panel, but, after 1 week of growth in medium containing 40% supernatant, there was high lytic activity against NK sensitive tumours but no lysis of NK resistant tumours (Fig. 1). Although mouse supernatant was used in this experiment, identical results were obtained in repeat experiments with rat supernatant.

To date we have observed the expression of NK activity in six different CTL clones. Some clones even express significant NK activity when cultured in 2% spleen cell supernatant, but this activity can still be amplified by culture in 40% supernatant. All the clones were originally obtained by limiting dilution at

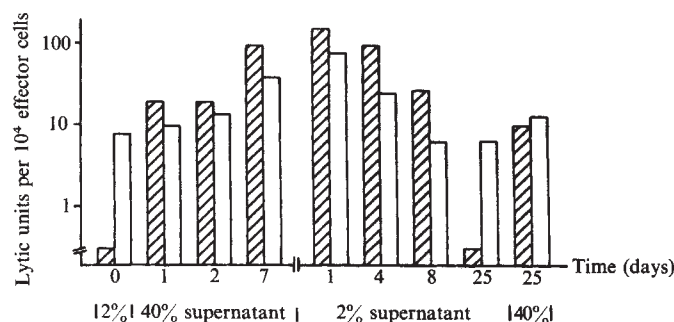


Fig. 2 The kinetics of gain and loss of NK reactivity in the k anti-b CTL clone, AIG7. On day 0, AIG7 cells were placed in medium containing 40% mouse spleen cell supernatant, and cytolytic activity was measured against YAC-1 (NK target, shaded bars) and EL4 (specific target, open bars) as a function of time. After 7 days, the cells were washed and transferred back into medium containing 2% rat supernatant, and cytolytic activity followed for a further 25 days. On day 23 an aliquot of cells was transferred for a second time into 40% supernatant. Cytolytic activity was calculated as lytic units per 10^4 effector cells, and is plotted on a logarithmic scale. One lytic unit is the number of effector cells required for 20% lysis.

1 or 0.5 cells per well and Poisson analysis showed that the probability of monoclonality for any individual clone was >90%. In addition, recloning of cell lines has produced daughter clones with the same propensity to express NK activity as the parental clones. In one case, further recloning of one of these daughter clones was undertaken. NK activity could be readily induced in all three 'grand-daughter' clones. Taken together, these observations rule out the possibility that the dual functional potential of CTL clones is due to a lack of monoclonality.

The kinetics of induction of NK activity in a specific CTL clone showed an initial sharp rise in NK activity during the first day of culture in 40% supernatant, with no change in activity against the specific target, EL4 (Fig. 2). During the next 6 days there was a continuing, but slower, rise of NK activity accompanied by a rise in activity against the specific target. At day 7, the cells were washed and replaced in 2% supernatant. During the next 8 days the high NK activity declined slowly, and almost disappeared by day 25. However, at this time, NK activity could be readily reinduced by transferring the cells back in to medium containing 40% supernatant. Thus, factors present in Con A induced spleen cell supernatant were able, selectively and reversibly, to alter the cytolytic specificity of CTL.

Preliminary studies to examine which factors in the Con A-induced spleen cell supernatant might be responsible for induction of NK activity in CTL have implicated both IL-2 and IFN. A typical experiment is shown in Fig. 3. Control CTL cultured in 2% rat spleen cell supernatant had no detectable NK activity (group a), but after 3 days culture in 30% mouse spleen cell supernatant high NK activity was found (together with some elevation of specific lytic activity). Addition of various amounts of Con A (0.3 – $10 \mu\text{g ml}^{-1}$) to 2% rat spleen cell supernatant induced no significant NK activity. Group c shows data obtained with $3 \mu\text{g ml}^{-1}$ Con A, equivalent to the maximum concentration of Con A that could be present in 30% spleen cell supernatant. The induced NK activity could thus not be explained as simple lectin-dependent cell-mediated cytotoxicity (LDCC). The observed failure of CTL incubated with Con A to display LDCC agrees with previous studies demonstrating that pretreatment of CTL with this mitogen fails to induce LDCC (see, for example, ref. 8). Indeed, incubation with Con A usually inhibited lysis by CTL (see group c), in agreement with a recent report⁹.

By contrast, incubation of CTL with partially purified IL-2 (free of detectable IFN) induced significant NK activity (group

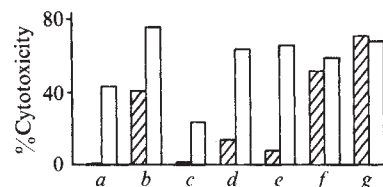


Fig. 3 Induction of NK activity by various mediators. Cytolytic activity was measured against YAC-1 (NK target, shaded bars) and EL4 (specific target, open bars) at an E:T ratio of 1:2. B6-C-H-2^{bml}/ByJ anti-C57BL/6 clone 3 cells were incubated for 3 days at 1×10^5 cells per ml in Click's medium: 10% FBS supplemented with the following: a, 2% rat spleen cell supernatant; b, 30% mouse spleen cell supernatant (containing 10 units/ml IL-2); c, 2% rat spleen cell supernatant + $3 \mu\text{g/ml}$ Con A; d, 10 units per ml IL-2. IL-2 was purified from mouse LBRM-33 tumour cells by successive ammonium sulfate precipitation, Sephadex G200 chromatography, and isoelectric focussing, as described elsewhere²⁴, and was kindly donated by Dr. S. Gillis, Immunex Corp. This IL-2 preparation contained no IFN detectable by inhibition of the cytopathic effect of vesicular stomatitis virus on mouse L929 cells; e, 10 units per ml IL-2 + $3 \mu\text{g/ml}$ Con A; f, 2% rat spleen cell supernatant + 10^4 units per ml mouse fibroblast IFN (Lee-Biomolecular Lab; g, 10 units per ml IL-2 + 10^4 units per ml IFN.

d), which was not augmented by Con A (group e). The extent of NK induction by the same batch of IL-2 has been variable between experiments, ranging from 0–100% of the activity induced by mouse spleen cell supernatant. The reason for this variability is currently unknown. Partially purified mouse fibroblast interferon was a potent inducer of NK activity (group f), and, in other experiments, induction of NK activity by this preparation of IFN could be completely neutralized by an antiserum to mouse Type 1 IFN. Treatment of CTL with a mixture of IL-2 and IFN gave the most potent induction of NK activity (group g, with considerably more activity than was induced by crude mouse spleen cell supernatant (group b)). IL-2 and IFN appeared to act synergistically: IL-2 alone induced 2 lytic units per 10^4 effectors, IFN alone induced 20 lytic units per 10^4 effectors, and the mixture of the two lymphokines induced 200 lytic units per 10^4 effectors (1 lytic unit being the number of effectors required for 20% lysis). By contrast, the activity against the specific target, EL4, was essentially constant between these three groups, at about 50 lytic units per 10^4 effectors. The apparent role of IL-2 and IFN as major inducers of NK activity in CTL is in agreement with the effects of these lymphokines on the NK activity of normal spleen cells^{10,11}. Interestingly, a synergistic effect of IL-2 and IFN on potentiation of splenic NK activity has been reported¹¹.

It is important to note that the lytic specificity induced in CTL clones by short-term culture in high concentrations of spleen cell supernatant was identical with that of splenic NK cells. A number of cloned murine cell lines displaying 'NK-like' activity have recently been described^{12–15}, but the specificity of these lines often differs significantly from that of splenic NK cells. For example, lysis of NK resistant targets such as P815, EL4, and LPS blasts is observed^{13–15}. It has been shown that such specificity can be induced in CTL clones by adapting them to an antigen-independent state of growth, an adaptation which causes profound changes in surface biochemistry¹⁶. These studies, combined with the findings described here, demonstrate that CTL can exist in various states; a resting state where they generally display only antigen-specific reactivity; an activated state where they acquire true NK specificity; and an antigen-independent state where the NK reactivity has degenerated into a promiscuous cytotoxicity.

In summary, this work has led to a number of important conclusions. (1) CTL are not necessarily terminally differentiated end cells, but can be induced to express new functional activities. (2) CTL are not restricted to specific cytotoxicity against target cells bearing the immunizing antigens. (3) Cells

of indisputable T cell lineage can express NK activity. This latter conclusion provides direct confirmation of a hypothesis formulated by Klein¹⁷. The relationship between NK cells and CTL is further reinforced by the finding that cloned CTL lines express low quantities of NK alloantigens¹⁸. It therefore appears likely that at least a proportion of the NK activity found in

spleen or peripheral blood lymphocytes is due to T lymphocytes differentiating along a pathway shared by CTL.

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Enhanced luminescence procedure for sensitive determination of peroxidase-labelled conjugates in immunoassay

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Present luminescence assays for horseradish peroxidase (HRP) have limitations. Here we report a novel procedure in which the HRP-catalysed luminescence of a cyclic hydrazide (such as luminol) is multiplied severalfold by the addition of a synthetic component of the firefly bioluminescent system, D-luciferin (4,5-dihydro-2-(6-hydroxy-2-benzothiazolyl)-4-thiazole-carboxylic acid). The specific enhancement of HRP-catalysed light emission from cyclic hydrazides should extend the sensitivities of luminescently monitored assays, which have already been shown to be as sensitive as those using radioactive labels^{1,2}. This procedure has been applied to the immunoassay of serum α -fetoprotein, thyroxine, digoxin, hepatitis B surface antigen, immunoglobulin E and rubella virus antibody.

Chemiluminescent and bioluminescent techniques have high potential for the quantitation of a wide range of clinically

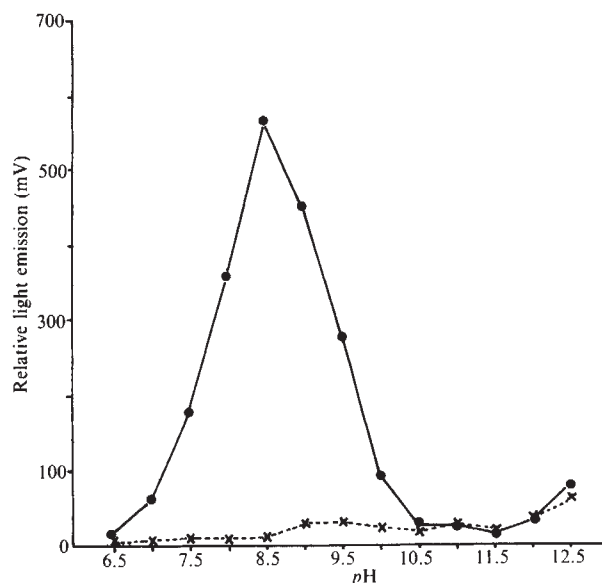


Fig. 1 Variation with pH of light emission from the HRP-catalysed luminescent reactions. Conditions were as described in Table 1 legend, with 0.1 M Tris-HCl buffer for pH 6.5-8.5 or 0.1 M glycine/NaOH for pH 9-12.5. ●—●, Luminol plus firefly luciferin; ×---×, luminol alone.

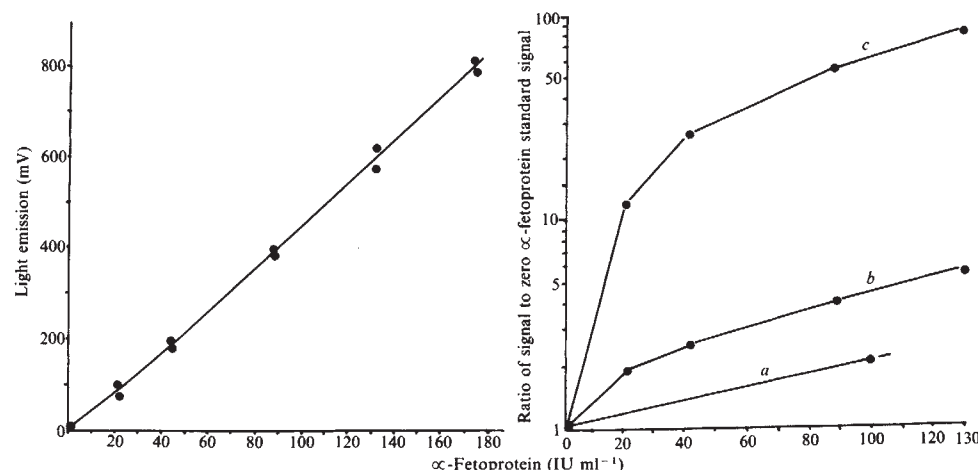


Fig. 2 Solid-phase immunometric assays for α -fetoprotein. Polystyrene spheres (6.4 mm) coated with rabbit anti-human α -fetoprotein antibody were incubated successively with serum samples and HRP-labelled rabbit anti-human α -fetoprotein antibody for 1 h at 37°C. After washing, residual HRP activity on the spheres was determined either colorimetrically by incubation for 0.5 h at 37°C with 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonate) (2 mM, pH 4.2), or by the luminescent systems described in Table 1. Left, standard curve with combined reagent at pH 8.0. Right, signal-to-background ratio for: a, luminol (pH 8.0); b, colorimetry; and c, luciferin plus luminol (pH 8.0) for different α -fetoprotein concentrations.

Table 1 HRP-catalysed light emission (with H₂O₂ as oxidant) from luminol alone, firefly luciferin alone and the combined reagents

	Light emission (mV)	
	Background	With HRP
Luminol (1.25 mM)	1.5	77
Firefly luciferin (39 µM)	<0.1	2
Luminol plus firefly luciferin	0.25	570

Aliquots of luminol, firefly luciferin, HRP-labelled antibody and H₂O₂ (2.7 mM) in 0.1 M Tris-HCl buffer pH 8.0, were mixed together to initiate luminescent reactions by injection of 0.9 ml of the same buffer. Light emission was monitored as photocurrent by using a luminometer constructed in this department, and recorded 30 s after injection. Measurement of total light emission by integration of the area under the emission curve produced similar results. Light emission was also enhanced if sodium perborate was used as oxidant, or with isoluminol or 7-dimethylaminonaphthalene-1,2-dicarboxylic acid hydrazide in place of the luminol.

tant analytes³. Catalysts of luminescent reactions have been considered as alternatives to radioactive labels in immunoassay systems but although they can be very sensitively detected by luminescence, activity is lost on conjugation and interfering substances in biological fluids reduce the sensitivity further. Horseradish peroxidase may be assayed by several luminescent procedures¹⁻⁷ and is less prone than other labels to loss of activity when incorporated into conjugates⁸. The limited availability of reagents such as *Pholas dactylus* luciferin⁶ precludes the routine clinical use of bioluminescent systems; chemiluminescent techniques suffer from greater susceptibility to interference, rapid decay of light emission and low light intensity in conditions in which interference is minimized.

During our investigations we have discovered an enhanced luminescence procedure for assay of HRP using readily available reagents. The procedure involves the simultaneous use of synthetic firefly luciferin (Sigma) and luminol or a luminol analogue at pH 7–10 in the presence of a suitable oxidant⁹. Total light emission from the luciferin and luminol analogue together significantly exceeds that from either substance alone, it also yields a greatly (up to 80-fold) improved signal-to-background ratio and increased specificity for peroxidase determination. The synergistic effect of luciferin on light emission from the peroxidase-catalysed oxidation of luminol is shown in Table 1, and the effect of pH in Fig. 1.

We have incorporated this enhanced reaction into a wide range of established immunoassay systems that use peroxidase conjugates and have thus increased their sensitivity. Levels of light emitted are high and relatively constant and the concentration of HRP can be estimated in seconds instead of the 0.5–2 h needed by colorimetry. Figure 2 illustrates the application of this method to an immunometric assay for α -fetoprotein and the improvement in sensitivity obtained. The sensitivity, speed and specificity of the technique can also be used in rapid immunoassay screening of specimens for viruses or bacteria. Preliminary results of screening sera for hepatitis B surface antigen by a test which can be completed in 30 min illustrate that such assays are feasible.

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Polymorphism near the rat prolactin gene caused by insertion of an *Alu*-like element

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Primate *Alu* and rodent *Alu*-like elements comprise major families of mammalian small dispersed repetitive DNAs. These elements are repeated more than 10⁵ times per haploid genome and are found between known genes, in introns and in satellite DNA¹⁻⁵. Their dispersion throughout the genome and the presence of directly repeated DNA sequences flanking the elements suggest, but do not prove, that they are capable of transposition. We describe here an allelic variation in the 5'-flanking region of the rat prolactin gene that offers the opportunity to examine the sequences of matching regions of two homologous chromosomes which differ in the presence of an *Alu*-like repetitive DNA element. Our findings support the hypothesis that these elements are integrated into the genome by generating short direct repeats of host DNA.

The prolactin gene of the rat has been shown to have a complex structure, with five coding exons extending over 10 kilobases (kb), with four large introns which include some regions of repetitive DNA⁶⁻⁹. A restriction enzyme map of the 5'-flanking region of several genomic clones revealed a dimorphic locus ~7.6 kb upstream from the first exon (Fig. 1C). One of the genomic clones (55) contained an additional 112 base pairs (bp) of DNA not present in two other clones, 11 and 17 (not shown). This additional DNA was repetitive, hybridizing to nick-translated total rat DNA (Fig. 1B). The surrounding sequences, represented in the 0.73-kb *Hind*III fragment of clone 11, appeared to be unique. They did not hybridize to total rat DNA, and hybridization of this fragment to total rat DNA revealed discrete bands (Fig. 3).

Figure 2 shows the sequence of the additional DNA from clone 55. The insert begins 154 bp 3' to the second *Eco*RI site shown in Fig. 1C. The structure of this segment of DNA is very similar to *Alu*-like repetitive DNA elements reported elsewhere¹. It is flanked by direct repeats, contains an A-rich segment at the 3' end, and includes sequences similar to the consensus promoter of RNA polymerase III¹.

Several *Alu*-like families have been described in rodents with some sequence homology to the primate *Alu* sequence¹⁻⁴. The inserted element described here shows considerable sequence homology to an insert in the rat α -tubulin pseudogene⁴ and part of the repetitive region in the fourth intron of the rat growth hormone gene³. It differs from that reported in the α -tubulin pseudogene only by single nucleotide deletions or substitutions with the exception of the absence of three Gs present in the pseudogene at the 5' end (Fig. 2).

The sequence of clone 11 contains one copy of the 11-bp sequence which forms the direct repeats on either side of the insert in clone 55. The remainder of the clone 11 sequence is identical to that of the regions flanking the insert in clone 55.

The similarity between the structure of *Alu*-like family members and pseudogenes has suggested that they might be formed by conversion of RNA intermediates to a cDNA which is integrated elsewhere in the genome¹⁰⁻¹². Transcripts homologous to *Alu* sequences have been described in a variety of tissues^{11,12} and RNAs containing a sequence homologous to that described here have been reported in rat brain¹³. Extrachromosomal DNA enriched with *Alu* sequences has been

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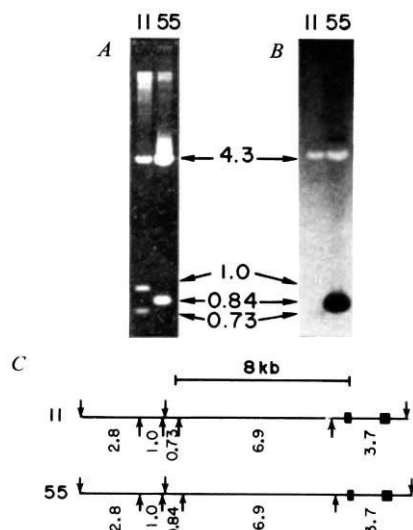


Fig. 1 Clone-specific repetitive DNA upstream from the rat prolactin gene. The 1.0- and 0.73-kb *Hind*III fragments from clone 11 and the 0.84-kb *Hind*III fragment from clone 55 were subcloned into pBR322. Recombinant plasmid DNA was cleaved with *Hind*III and the fragments separated on a 1.0% agarose gel (A) before transfer to nitrocellulose⁹. The filter was prehybridized at 68 °C in 0.90 M NaCl, 90 mM Na-citrate pH 7.0, 0.50% SDS, 0.020% each of Ficoll 400, polyvinyl pyrrolidone (PVP) and bovine serum albumin (BSA) for 30 min and in hybridization buffer (0.45 M NaCl, 45 mM Na-citrate pH 7.0, 0.10% SDS, 20 mM Na-phosphate, 50 μ g ml⁻¹ poly(A) and 0.20% each of Ficoll 400, PVP and BSA) for 2 h. The filter was hybridized in 5.0 ml of hybridization buffer with 50 μ g ml⁻¹ denatured herring sperm DNA for 15 h at 68 °C. The probe was 1 μ g of total rat DNA labelled with ³²P by nick-translation to 8×10^7 c.p.m. μ g⁻¹. After hybridization, the filter was washed at 60 °C in 0.30 M NaCl, 30 mM Na-citrate, 25 mM Na-phosphate pH 7.0, 5.0 mM EDTA, 0.10% SDS, 1.5 mM Na-pyrophosphate and in 0.15 M NaCl, 15 mM Na-citrate pH 7.0, 5.0 mM EDTA, 0.10% SDS before exposure to Kodak XAR-2 film with intensifying screen at -80 °C for 90 min (B). Maps of the pertinent portions of clones 11 and 55 (C) show *Eco*RI (↓) and *Hind*III (↑) sites as well as fragment sizes (in kb) and the first two prolactin gene exons (solid boxes).

EcoRI

aattcactaa aaaacattca ggctttcaca gtttaacaag caatttggtg taacactcta attttggtga
cacaaaagt tataaaagt ttaattgaca atttgaggtt gagttaacaa gggttgtaat gttttcaca
aaatgccta atctcctggc TGGCNC ACTGG
GATTTAGCTC AGCGTAGAGC GTTGCTAGC AACCCGAGC CCGTCTGC
ANNCC
GTCGCCAGCT CCGAAAAA AAAAAAAA AAAAACCA AAAAATCGGC TAATCTtat agtactctgc
tcaagtga ctattatcat ttgattctag tggaaatgc ttaagtatt ggaggatc
Sau3A

Fig. 2 Nucleotide sequence of clone 55 from the *Eco*RI site within the 0.84-kb *Hind*III fragment (Fig. 1C) to the *Sau*3A site 338 bp 3' to the *Eco*RI site. The *Eco*RI-*Sau*3A fragment was cloned between the *Eco*RI and *Bam*HI sites of M13mp8 and mp9 (ref. 16), and both strands sequenced by the dideoxy chain termination technique¹⁷. Lower case letters give the sequence common to both clones 11 and 55; the sequence inserted into 55 is in upper case letters. The direct repeats are underlined. Nucleotides differing from the sequence analysis of an *Alu*-like family member found in a pseudogene for α -tubulin⁴ are indicated by asterisks; sites of nucleotide deletion are designated by an upward arrow. The split consensus sequences for an RNA polymerase III promoter¹⁸ are given above the repeat element sequence.

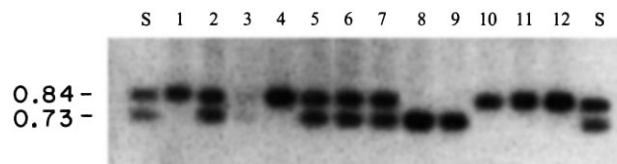


Fig. 3 Allelic distribution of the insert. DNA was isolated from the livers of 10 individual Holtzman female rats (lanes 1-10) and also from the brain (lane 11) and kidney (lane 12) of the tenth rat⁹. Portions (20 μ g) of each sample were digested with *Hind*III, and the fragments separated on a 1.3% agarose gel. After transfer to a nitrocellulose filter, the DNA was hybridized (see Fig. 1 legend) to 200 ng of the 0.73-kb *Hind*III fragment from clone 11 labelled with ³²P by nick-translation to a specific activity of 10^8 c.p.m. μ g⁻¹. The filter was then washed and placed in contact with X-ray film with an intensifying screen for 6 days. The lanes marked S contained equal amounts of DNA from clones 11 and 55 diluted with a herring sperm DNA solution and digested with *Hind*III.

reported, suggesting that these might be involved in the transposition event¹⁴.

The dimorphism described above has also been found in clones of genomic DNA prepared from the liver of one Sprague-Dawley rat¹⁵. To examine the distribution of this allele, liver DNA from 10 Holtzman rats (Sprague-Dawley strain; Holtzman Company, Madison, Wisconsin) was examined by restriction enzyme digestion and hybridization to the 0.73-kb *Hind*III fragment of clone 11 (Fig. 3). Half of the animals examined were heterozygous at this locus, three were homozygous for the allele with inserted DNA, and two were homozygous for the allele without the insert. Brain and kidney DNA from rat 10 gave identical results to that observed for the liver of that animal. In contrast to the outbred Holtzman rats, DNA from eight inbred Fischer 344 rats did not contain the insert.

The evidence presented here supports the mobility of *Alu*-like repetitive elements in the rat. However, it is unknown whether these sequences are presently moving about the genome, or if their movement was restricted to a specific evolutionary period. Considering that about 10^5 *Alu* or *Alu*-like sequences are distributed through the mammalian genome, it is probable that there exist many allelic differences like that described here. Such differences may not have been previously reported due to examination of cloned genes from just one chromosome, or the small change in mobility of large DNA fragments on agarose gels caused by these short elements. If these differences are common, they should be useful genetic markers for mapping genes on chromosomes, and for analysing individual lineages.

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Organic grains in Taurus interstellar clouds

WHITTET *ET AL.*¹ have reported the detection of an interstellar absorption feature over the 2.9–3.9- μm waveband arising from dust grains in the Taurus clouds. The absorption is found to peak at the wavelength $\lambda \approx 3 \mu\text{m}$, with an extended shoulder on the longwave side of the peak, implying an additional broad absorption band centred on $\lambda \approx 3.4 \mu\text{m}$. Whilst the absorption at $3 \mu\text{m}$ could be attributed to OH stretching in H_2O -ice the broad absorption at $3.4 \mu\text{m}$ must arise from CH stretching in some form of condensed organic material. If τ_3 and $\tau_{3.4}$ denote the respective optical depths at the centres of the 3- μm and 3.4- μm bands, Fig. 3b of ref. 1 gives a ratio

$$\frac{\tau_{3.4}}{\tau_3} \approx \frac{1}{4.8} \quad (1)$$

for the Taurus material. Now, the mass absorption coefficient of ice at the centre of the 3- μm band, from the data of Bertie *et al.*², is

$$\kappa_{\text{ice}} \approx 36,000 \text{ cm}^2 \text{ g}^{-1} \quad (2)$$

using a specific gravity $s = 0.917$ for ice. (For amorphous ice³ the same mass absorption coefficient is obtained for a slightly lower value of specific gravity, $s = 0.75$.) For organic polymers of a wide range of types, including polyformaldehyde^{4,5} and biomolecules⁶, we find that the absorption coefficient κ_{org} at the centre of a broad 3.4- μm band satisfies the inequality

$$\kappa_{\text{org}} < 1,200 \text{ cm}^2 \text{ g}^{-1} \quad (3)$$

Noting that

$$\frac{\tau_{3.4}}{\tau_3} = \frac{\kappa_{\text{org}} \rho_{\text{org}}}{\kappa_{\text{ice}} \rho_{\text{ice}}} \quad (4)$$

where ρ_{org} and ρ_{ice} are the respective mass densities of organic material and of ice, and using equations (1), (2) and (3) we obtain

$$\frac{\rho_{\text{org}}}{\rho_{\text{ice}}} > 6.25 \quad (5)$$

We thus conclude that there is a clear preponderance of condensed organic matter over H_2O -ice in the Taurus dust clouds.

Note added in proof: We are well aware of the references cited in the annexed reply, but consider them irrelevant. Interstellar grains cannot be largely H_2O -ice of radii $0.3 \mu\text{m}$ because then the extinction

at $5,000 \text{ \AA}$ would be comparable to that at the centre of the 3- μm band, whereas observationally it is at least an order of magnitude more.

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WHITTET REPLIES—The conclusion drawn by Hoyle and Wickramasinghe must be treated with caution, as it is based on the assumption that C–H bond absorption in organic polymers is the only possible explanation of the long-wavelength ‘shoulder’ in the 3.1- μm interstellar ice band profile. As pointed out in our paper¹, several other explanations have been put forward. Leger *et al.*² have argued that the phenomenon is a scattering effect in large ice grains, and that the entire profile can be matched by H_2O ice containing a small proportion of NH_3 . Recent model calculations by Tielens and Hagen³ suggest that grain mantles contain $\sim 60\%$ water-ice. These^{3,4} and other⁵ investigators attribute the shoulder of the ice feature to hydrogen bond interactions between H_2O and other molecules such as NH_3 , H_2CO and H_2O_2 in the icy matrix. There is clearly no need to invoke complex organic molecules to explain this feature; moreover, the case for organic polymers as major grain constituents has been weakened by recent results⁶ concerned with other parts of the spectrum.

Present astronomical data at $3 \mu\text{m}$ are of insufficient spectral resolution to allow firm conclusions to be drawn on the detailed chemistry of interstellar grain mantles. Recent advances in instrumentation offer the prospect of major advances in this field in the near future.

Note added in proof: We have not attempted to explain the total visual extinction in terms of pure ice grains; indeed, our results imply that ice only appears at optical depths $>$ about 5 magnitudes into the cloud. The authors cited above (refs 3 and 4) have demonstrated that a core-mantle grain model in which the mantle, where present, is predominantly water ice, can match the 2.9–4.0 μm absorption profile. We have no

reason to doubt the integrity of these results.

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ESR studies of planktonic foraminifera

SATO¹ has recently reported the possibility of dating foraminifera by ESR techniques. We wish to comment on three problems relating to this method of dating: first, the signal may be reduced by optical bleaching during laboratory handling; second, the dose contribution due to excess ^{230}Th must be taken into account; third, the signal may be unstable for samples greater than a million years old because of thermal decay, rather than anomalous fading as he suggested.

As part of a project on the ESR and thermoluminescence (TL) properties of foraminifera, we have made a brief study of the effect of light on the TL signals. Although stalagmitic calcite has been reported to be relatively insensitive to light², we are very much aware of the light sensitivity of other materials³. We took a portion of foraminifera $> 60 \mu\text{m}$ from the top of a deep ocean core, RC14-37 (lat. $01^\circ 28' \text{N}$, long. $90^\circ 10' \text{E}$) and gave it a γ dose of 600 Gy (60 krad). Subsamples were then placed 7 cm from a 40 W tungsten lamp for 15 h and others placed 35 cm from a 300 W mercury sunlamp for 7.5 h . The samples lost respectively 78 ± 2 and $95 \pm 2\%$ of the signal from the 275°C TL peak. Since the electron traps responsible for the ESR signal are thought to be the same as those giving rise to the 275°C TL peak⁴, we think that experiments should be carried out to determine whether the ESR signal is similarly affected by light. If so, appropriate precautions should then be taken when handling samples to be used for dating. It is, of course, possible that the loss of TL signal could be due to a reduction of the number of active luminescence centres, in which case no loss of ESR signal would be seen.

We also obtained the equivalent dose (ED) for two very old samples of foraminifera; a 2.6-Myr sample V20-163 (75 cm)⁵ and a 3.2-Myr sample DSDP Site 357 (2/5/10-13)⁶. The EDs were obtained experimentally by the additive dose method, which was also used for the ESR measurements by Sato¹. The TL was measured using a Daybreak TL system with a Corning 4-69 filter (50% transmission between 340 and 580 nm) between the heat absorbing filter and the EMI9635 photomultiplier tube. The TL peak obtained at 275 °C for a heating rate of 5 °C s⁻¹ is typical of pure calcite². The foraminifera from V20-163 had already been cleaned and hand picked in ordinary room fluorescent lighting for oxygen isotope analysis but those from DSDP357 were prepared in the dark. They gave ED values of 110 and 250 Gy (11 and 25 krad) respectively.

To see whether an ED of 250 Gy was reasonable for the foraminifera extracted in the dark, radioactivity analyses were carried out to calculate the annual dose rate. α counting⁷ of DSDP Site 357 bulk sample gave a total count rate of $0.052 \pm 0.004 \text{ cm}^{-2} \text{ ks}^{-1}$ which predicts an annual dose rate of 140 μGy (14 mrad). For high carbonate cores, the potassium content is inversely related to the carbonate content⁷ and using the value for an 85% carbonate core⁶ ($\text{K}_2\text{O} = 0.2\%$) we get an additional annual dose rate of 70 μGy . The cosmic dose rate is negligible under several kilometres of ocean. These data predict a total dose of 630 Gy (63 krad) for a 3 Myr sample. An additional dose due to the decay of excess ^{230}Th must also be included. This was ignored by Sato¹, even though his cores are beneath 4 km of ocean water. α counting of RC8-39⁷, a core at a similar water depth (4.33 km), gave a core top count due to excess ^{230}Th of $1.8 \text{ cm}^{-2} \text{ ks}^{-1}$ in agreement with α spectrometric measurements. This excess activity will produce an additional ED of about 500 Gy (50 krad) for any sample in the core older than 0.3 Myr. Indeed, this component would give rise to exactly the type of behaviour shown in Fig. 3 of ref. 1.

The ED obtained from TL measurements (250 Gy) is thus considerably less than that predicted from radioactivity data (1,130 Gy). We therefore think that the electron trap responsible for the 275 °C peak may not be sufficiently stable for dating samples as old as 3 Myr. Similar low values of ED were obtained by Sato¹ for samples older than 1 Myr. Sato assumed that the radiation defects responsible for the ESR ($g = 2.003$) signal that he used for dating were the same as those of the 275 °C TL peak, as found experimentally by Valladas *et al.*⁴. Although the 275 °C peak has been attributed with a mean life of $\sim 10^8$ yr at

10 °C (ref. 8), more recent kinetic studies on calcite (N. C. Debenham, personal communication) give a minimum mean life of ~ 1 Myr. Hence the trend towards a constant value of dose with depth may be due to thermal decay of the defects at ambient temperature, not anomalous fading as he suggested. Another possibility is that diagenetic changes may have occurred in such old foraminifera (N. J. Shackleton, personal communication). Bothner and Johnson⁹ also found a saturation of the TL signal with depth. They studied the TL response of foraminifera $> 74 \mu\text{m}$ relative to the α activity down a number of cores and found that the thermal decay constant for the TL was $\sim 10^{-6} \text{ yr}^{-1}$ such that the mean life at ocean bottom temperatures was $\sim 10^6$ yr. They also concluded that this was lower than that obtained from TL studies of limestone. Further ESR and TL studies on constantly deposited foraminifera should produce a more precise determination of the mean life for calcite in the natural environment. This is of wider interest since attempts are being made to use ESR to date stalagmites and bones associated with fossil hominids.

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SATO REPLIES—The ESR signal of foraminifera may be reduced by optical bleaching, as Wintle and Huntley pointed out, if the samples were stored in light. I prepared two groups of samples consisting of foraminifera of diameter 149–250 μm and 250–503 μm , and gave them a γ dose of 40 krad. The ESR signal was reduced by $31 \pm 3\%$ and $24 \pm 3\%$, respectively, using a 7-h exposure to a 100 W tungsten lamp placed 7 cm from the samples. Although reproducibility of the signal intensity shows that the bleaching by short exposures to ordinary room fluorescent

lighting is negligible, the samples to be used for dating should be prepared from sediment which has not been exposed to light for a long time.

γ -ray spectrometry of 11 samples taken from core KH 73-4-7 was performed by M. Sakanoue and K. Komura (personal communication). The equivalent content of ^{238}U decreases rapidly with depth from 13.46 p.p.m. at a depth of 6 cm to 0.24 p.p.m. at 140 cm due to the excess of ^{230}Th and then remains nearly constant. The mean equivalent content of ^{232}Th was 1.67 p.p.m. and the mean potassium content was 0.56%. The annual dose predicted from these data decreases with depth from 0.4 rad yr^{-1} to 0.1 rad yr^{-1} if the effectiveness of α -radiation for inducing ESR signal is assumed to be zero. It is improbable that the large contribution of excess ^{230}Th to the annual dose is completely compensated by insufficient exposure to γ radiation near the sediment–water interface¹. On the other hand, the rate of decrease in the content of U-series may predict a slow sedimentation rate (3.2 mm kyr^{-1}) for the uppermost 70 cm. In future work, it will be necessary to estimate the effect of reduced exposure near the surface of the sediment.

As there is little information about the stability of the signal in foraminifera, further studies on uniform and constantly deposited sediments are needed to evaluate the mean life time.

I thank Professor M. Sakanoue and Dr K. Komura for valuable information on the content of radioactive elements.

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Matters Arising

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Cause for concern

Eric Ashby

Victims of Science: The Use of Animals in Research, revised edn

By Richard D. Ryder.

National Anti-Vivisection Society Ltd, London: 1983. Pp.180. Hbk £6.75, pbk £3.75.

NEARLY 20 million motor vehicles are licensed to run on the roads of Britain. Consider what it would be like if this traffic were to be controlled by an Act of Parliament, never amended, dating from the 1870s, a decade before the internal combustion engine was invented, when there was nothing more dangerous on the roads than horses. A silly speculation? No sillier than the plain fact about the Act of Parliament that controls experiments on animals.

The Act was passed in 1876, with the prime object of deterring a few scientists from carrying out surgical experiments on conscious animals. Some of those experiments, by all accounts, were horrifying to witness and of dubious scientific value. There were not many of them — a few hundred a year — and, to put the matter in perspective, it has to be remembered that less than ten years before that Act was passed it was still legally permissible in America to own slaves. Today this same Act, unamended, is used to control over 4 million procedures on animals each year. Only a minority of these procedures is surgical; an even smaller minority subjects the animal to pain, for the animals are put under anaesthetic and are killed before they regain consciousness if otherwise they would suffer severe pain.

In a typically British pragmatic way, the Home Office inspectors who supervise the 1876 Act have devised a working relationship with those to whom licences are granted to ensure that animals are treated "with due care and humanity". Though the Act is obsolete its interpretation is not. It covers procedures undreamt of by those who campaigned in the 1870s for a law to protect animals against abuse from a handful of physiologists and anatomists.

Notwithstanding this liberal and humane interpretation of an obsolete law, the law needs to be changed. It does not, for instance, cover procedures on animals which are not 'experiments', i.e. procedures not designed to test a hypothesis, but (for example) to be used for the preparation of a vaccine. Nor does the law control the housing of animals to be used for experiments, nor the sources from which they come. Nor does it take sufficient account of the purpose of an experiment, i.e. whether it is likely to benefit the welfare of human beings or animals. So a campaign to repeal the 1876 Act and to replace it by a law which gives better protection to animals has been under way for some time. It has already 'brought animals into

politics': the manifestos of all political parties include a promise to revise the law; the Council of Europe (at a glacial pace) is drawing up a convention to which member states will be asked to subscribe; the British government issued in May 1983 a White Paper (a document of intent) for an updating of the 1876 Act.

Among the campaigners who have succeeded in stirring the conscience of the public over this issue is Richard Ryder. This book is a revised edition of his case studies "of the way man mistreats animals for the purposes of research". It is a catalogue, a very selective one, of the uses to which animals are put in medical and non-medical research. It contains some useful facts and figures, and — its chief virtue — a clear account of the campaign for reform, from the beginning of the 19th century up to the present. The book is illustrated by lurid coloured plates of animals undergoing experimental surgery, drawn, for some unexplained reason, from Japan, Poland, Mexico, Spain, the Soviet Union, and the USA, not from Britain.

The purpose of Ryder's book is not only to stir the public conscience; it is also (in his own words) "to suggest reforms". It is not Ryder's fault that this is the weakest part of the book, for in the present state of knowledge, the only reform that would satisfy zealots in the animal rights movement would be to discontinue most medical and veterinary research, to abandon the testing of drugs, pesticides, and food-additives before they are put on the market and to



Public concern — a demonstration in Cambridge in 1979.

discontinue the raising of cattle, sheep, and poultry (except for their eggs) in favour of vegetarianism. Obviously the zealots are not going to be satisfied. What, then, is the best way, *in the interests of animals*, to reach an understanding between those who want to protect animals from exploitation on one hand, and on the other hand those who believe that it is essential that, for some purposes, and "with due care and humanity", animals have to be exploited?

Not, in my view, through books like *Victims of Science*. For one thing, the title is totally misleading. The great majority of animals are not victims of science: they are sacrificed to meet the public demand for safety, not to advance science but to make sure that drugs will not have nasty side effects, that substitutes for sugar will not cause cancer, that mouth washes will not irritate the skin. The animals are victims of the consumer society and it is a pity Ryder — who makes that point clearly in the text — wasn't more precise about the title.

There are other weaknesses about the book. Ryder seems confused about the attitude one should have to medical, as contrasted with non-medical, research. On one page he emphasizes this contrast, as though the use of animals for medical research is, so to speak, a special case. On another page he seems to dismiss the contrast, saying that the only justification for inflicting pain on any animal is that "it must be in terms of benefits accruing to the *same* individual". How much more persuasive he would have been, in the long run, if he had admitted that all of us — animal-protectors and animal-exploiters alike — are up against a daunting ethical problem that has not been solved. Unfortunately Ryder omits from his bibliography, which is disappointingly partisan, some of the thoughtful and sympathetic writing about this dilemma. Why, for instance, is there no reference to Marian Dawkin's book on *Animal Suffering*? Or (though casually mentioned in the text) to the 340 pages of evidence given to the Select Committee on the Laboratory Animals Protection Bill? Or to the thoughtful essays by Mary Midgley?

Perhaps in the short run sensational advocacy pays off. It could be claimed that the protection of animals, like the protection of the environment, has been promoted by scandalous incidents publicized in the media. But in the long run it is even-handed honesty that pays. In some of his writing, and in his evidence to committees, Richard Ryder has presented a convincing and well argued case. In this book, however, he is preaching a necessary message — that we should continue to worry about the use of animals for the benefit of human beings — in an unnecessarily shrill voice. □

Lord Ashby was chairman of the House of Lords Select Committee appointed to report on the Laboratory Animals Protection Bill in 1979.

The Publics of public health

William Coleman

Endangered Lives: Public Health in Victorian Britain.

By Anthony S. Wohl.

J.M. Dent: 1983. Pp.440. £17.50.

OVER the past forty years public health in Victorian Britain has remained a subject of uncommon historical interest and has generated a large body of valuable scholarship. The scope of the subject is immense, involving virtually the full range of biological, environmental, economic, political and social conditions that can influence human well-being and the documentary material, available for assaying the character of these conditions, is almost overwhelmingly abundant and diverse. Is there a way through this labyrinth? How can one begin to appreciate the multiple and inter-related meanings of the critical word "public"? (The meaning of "health" is itself another and vast problem, not addressed directly in this book.)

With singular boldness Anthony S. Wohl has attempted to create an integrated and critical portrait of the public health affairs of the age. He draws upon and makes imaginative use of available scholarly material (his references provide an excellent guide to relevant publications) and works also with the inexhaustible resources of Parliamentary Reports, other govern-

ment publications and contemporary general and medical literature. This is not the first such synthetic account (F.E. Smith's *The People's Health* should be mentioned) but Wohl's work stands out for its comprehensiveness and clarity of presentation. The latter is much assisted by the author's mastery of his material and his willingness to explore the diverse and not always immediately obvious aspects of a problem (see, for example, his discussion of infant mortality in relation to class standing, the plight of the working mother, breast versus bottle feeding, the obscure causes of infant diarrhoea and the persistence of the problem into the twentieth century).

The organization of *Endangered Lives* is simple and effective. After describing the condition of child and adult in the industrial city (Wohl does not neglect, importantly, the much less familiar misery of rural life), he discusses sewerage, fevers, overcrowded housing and man's fine capacity for fouling his nest, thereby either shortening his life or rendering it altogether nasty. Public action and inaction, too, are examined, with stress upon conflicts between local and central governments and the agonizing process of legislative innovation and even slower implementation of remedial measures.

There are many "publics" in this story: officialdom at various levels and representing rival interests; the producers and consumers of society's offal, the latter being especially the true subject of the word "public"; the working class; the medical profession, whose interest occasionally favoured closer scrutiny and concern for the labouring poor but often did not. Public health was, and is, therefore, a matter for neither legislator nor physician alone but one that reaches over the population and most crucially affects society's economically and politically most impotent members. Wohl stresses this point (but also notes the absence of working class agitation regarding specific public health matters); it is, in fact, the life of the poor or marginally secure that is the central subject of his book. This is an outstanding work of thoughtful synthesis and a paperback edition in the near future, would be very welcome. □

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Entropy, evolution and Cheshire cats

John Lawton

Adaptability: The Significance of Variability from Molecule to Ecosystem.

By Michael Conrad.

Plenum: 1983. Pp.383. \$42.50, £29.75.

MOST biologists are interested in the adaptations of organisms; very few will find those interests reflected in Conrad's book. Indeed, my guess is that most of them will find it extremely difficult, if not impossible, to understand. I have several reasons for coming to so critical a conclusion.

The book attempts to review patterns of adaptive change in living things, ranging from molecules to whole ecosystems. However, the basic concepts are formally developed using thermodynamics and information theory. The result is often a dense, impenetrable string of equations with components defined many pages earlier, but with no appendix to remind the struggling reader what each variable means. If hard ideas are unavoidable, notation is not something that readers should have to wrestle with.

Second, Conrad's style when dealing with difficult ideas does not help. I had the curious experience of reading sentences in which individual words were perfectly understood, but where the whole conveyed no meaning. Consider the following: "For adaptability the main relevant point is that biological systems are capable of coping with disturbances if they are capable of forgetting these disturbances. In general forgetting disturbance means that disturbance is ultimately dissipated into a heat bath". Quite so, but what does it mean? Or "The idea of a partial state is a little like the smile of the Cheshire cat, except that we are eventually interested in finding the cat". I wonder if Conrad really intended to draw so close an analogy with Alice's bizarre imaginary world? Of course, it is perhaps unfair to take bits of text out of context but in context, I often found his ideas no easier to comprehend.

If my main problem with the book was unfamiliarity with its basic ideas, it was compounded by a failure on the part of the author to convince the hapless reader that it would all be worth it in the end. It is not at all obvious, for example, why "statistical characteristics of the ecosystem are fundamental from the standpoint of adaptability". Yet two early chapters plunge straight into descriptions of these statistical characteristics in terms of entropy measures of transition probabilities of ecosystem states. To the working biologist, the sheer impossibility of ever measuring transition probabilities between ecosystem states defined over the whole hierarchy of levels from populations

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

UTILE CUM DULCE.

Inquisitive Gent. "YOU WILL—A—THINK ME VERY INDISCREET—BUT I CANNOT HELP WONDERING WHAT THIS ELABORATELY-CARVED AND CURIOSLY-RAMIFIED STRUCTURE IS FOR. IS IT FOR ORNAMENT ONLY, OR INTENDED TO HEAT THE HOUSE, OR SOMETHING?"

Fastidious Host. "O, IT'S THE DRAINS! I LIKE TO HAVE 'EM WHERE I CAN LOOK AFTER 'EM MYSELF. POOTY DESIGN, AIN'T IT? MAJOLICA, YOU KNOW. . . HAVE SOME CHICKEN!"

7. Domestic sewers. The guest's horror was probably prompted not so much by the indelicacy of the dinner conversation as by the fear, commonly held, of noxious vapours escaping from the sewer pipes.

Source: *Punch*, 6 January 1872

to cells (think of the number of cellular states in an ecosystem of even moderate complexity!) makes serious reading impossible. But if these ideas are not ultimately to be tested, what are they for?

Another disturbing feature of *Adaptability* is the apparently innocent way in which it runs across the mine fields of group selection, species selection and ecosystem selection in their many and arguable forms. Statements such as "Some traits might only be mildly disadvantageous to the species", "The community may use its adaptability to buffer the controllable environment from the uncertain perturbations of the uncontrollable environment" and "ecosystems will tend to

develop in directions which eliminate . . . unnecessary cost for the biota" will raise Darwinian hackles, yet appear throughout the text. If Conrad realizes that these are contentious statements he gives precious few indications of the fact.

Was the struggle worth while? The honest answer is "no". When I finished the text, I was no wiser about "The significance of variability from molecule to ecosystem" than I was when I started. I have a sneaking suspicion that there may be some novel and worthwhile ideas hidden in there, but like the Cheshire cat, they are awfully difficult to find. □

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Out on a limb

T.M.L. Wigley

Past Climates: Tree Thermometers, Commodities and People.

By Leona Marshall Libby.
University of Texas Press: 1983. Pp. 142.
£21.25, \$25.

THE cover design is excellent; a negative picture of a section of tree on a tasteful beige background, with the main title *Past Climates* arrestingly clear. But don't be misled. You'll learn precious little from this book about past climates, for the pages give only a highly personal view of two avenues of research into climate reconstruction that the author has explored. The restricted scope of the book is described in its subtitle. A chance to learn about dendroclimatology (*Tree Thermometers*) and about the effects of climate on society (*Commodities and People*), perhaps? Wrong again.

The first half of the book describes Libby's attempts to extract information about past climatic change from the stable isotopic composition of accurately dated wood samples. Some of this work was originally published in *Nature*, a sad comment on the fallibility of the refereeing system. Libby was one of the early workers in this field, but her work and results are viewed with considerable scepticism by other isotope climatologists. I thought that here Libby might be able to justify her particular approach, point out where other workers have gone astray, or reconcile the differences of opinion which exist. But no. None of the recent advances in our understanding — through the work of Ferhi and Letolle, Stuiver and Burk, Francey and Farquhar, Gray and Thompson, and others — is mentioned. All other work is summarily dismissed on the grounds that the analyses are based on "wet chemistry".

Libby adheres to her semi-empirical approach and all her book succeeds in is to document its inadequacies, errors and oversimplifications. As an example, consider her suggestion that the isotopic

composition of local precipitation is related to sea-surface temperature in the water vapour source region, and to local temperatures. Both suggestions are wrong, and it certainly can't be both. In attempting to support the suggested link Libby only considers the similarity of the seasonal cycles of precipitation $\delta^{18}\text{O}$ and temperature — an irrelevant comparison. Although the link with local temperatures is one which is commonly assumed, it is not confirmed by an analysis of IAEA data on precipitation $\delta^{18}\text{O}$ values. In general, interannual $\delta^{18}\text{O}$ variations do not correlate with interannual variations in local temperature.

Some of Libby's data do show vague trends which are similar to changes in temperature over the same period. For example, she compares German oak data with winter temperatures (in England) and Californian sequoia data with summer temperatures. (The argument in the latter case is that sequoias grow mainly in summer, so I suppose the oak must be assumed to grow in winter.) In none of these comparisons are any quantitative correlations calculated. Rigorous statistical testing of hypotheses is eschewed in other aspects of her work. For example, a purported relationship between normalized wheat prices and temperature turns out to be insignificant if due allowance is made for the effects of autocorrelation.

The final section of the book, "Human Interaction with Climate", I found to be interesting, but, in common with many other similar works on this subject, generally unconvincing. It is all too easy to interpret parallels in the timing of social and climatic events (both of which tend to be poorly dated) in a deterministic sense; and while such interpretations may be provocative they are usually scientifically unsatisfying.

Overall, this book has little to offer to the discipline of climatology. It must be one of life's small ironies that the author's name is an anagram of "alone". In this field, she is. □

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Fertile matter

R.J. Aitken

Reproductive Physiology of Vertebrates. 2nd edn

By Ari van Tienhoven.
Cornell University Press, Ithaca and
London: 1983. Pp. 560. £42, \$49.50.

As a young graduate tackling the complexities and bewildering diversity of vertebrate reproduction, I was frequently grateful for the presence of Ari van Tienhovens' *Reproductive Physiology of Vertebrates* (that is, until it was permanently borrowed by a colleague who clearly shared my appreciation of Van Tienhoven's ability to précis a vast literature). The recent issue of an updated and improved version of this impressive work is therefore to be welcomed, not only because it will replace an eight-year-old gap in my bookshelf but also because it will benefit a new generation of biology graduates seeking to increase the depth and breadth of their understanding of vertebrate reproduction.

It is in the scope of the text that the uniqueness and importance of this book lies. The list of species and topics covered is comprehensive, taking us from cyclostomes to humans on a range of subjects, covering the gamut of reproductive phenomena. The layout of the book is logical and an improvement on the first edition in many respects, incorporating new chapters on such key subjects as puberty, intersexes and immunological aspects of reproduction. The comparative nature of the text is also backed up by helpful diagrams and especially tables, many of which summarize volumes of data.

From the text one gains an insight into the lability of the reproductive process which can apparently adapt, in an infinite variety of ways, to meet the particular requirements of a species. Fascinating examples of this diversity include the 'delayed implantation' exhibited by certain mammalian species (such as roe deer, skunks, polar bears and wallabies) in which the embryo is held in a dormant state within the uterus for months on end, only to be suddenly activated at a time which will help ensure the subsequent survival of the young.

Even more bizarre is the phenomenon of gynogenesis exhibited by certain species of fish, of which only female forms exist. Having dispensed with the need for males, such species reproduce by annexing the spermatozoa of other teleost species to penetrate and initiate the parthenogenetic development of their ova.

No man can be an expert in all things however and, given the range of material reviewed by the author, it is understandable if small inaccuracies creep in, such as the notion that sperm-oocyte interaction does not exhibit species specificity or that

pronuclear fusion occurs in mammals. It might also be noted that the data surveyed by Van Teinhoven takes us up to 1980–81 and clearly in the most rapidly developing areas, such as testicular physiology, some of the concepts presented may be dated or incomplete.

The book does not, however, set out to cope with the sort of ephemerae found in symposium volumes. It is intended to be an introduction through which students and researchers entering the field of reproduction might gain a perspective on this process as it occurs in vertebrates; in this respect, it admirably succeeds. □

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Getting around

J.R.S. Fincham

Mobile Genetic Elements.

Edited by James A. Shapiro.
Academic: 1983. Pp. 688. £43, \$65.

DURING the past decade the orderly world of cytogenetics has been increasingly disturbed by the intrusion of a miscellaneous and apparently unruly collection of mobile genetic elements. Their influence has grown to the point where knowledge of their prevalence and properties is essential to anyone wishing to keep abreast of current thinking about such important topics as spontaneous mutation, genome evolution and oncogenesis. The time was certainly ripe for an authoritative account of movable sequences, and James Shapiro has organized one. He could hardly have assembled a better team of authors. All are in the forefront of research in their respective areas and all write well, with a real attempt to make their chapters mutually relevant.

Approximately equal weight is given to prokaryotic and eukaryotic systems. Four chapters are concerned with freely roaming prokaryotic sequences: Iida, Meyer and Arber deal with bacterial insertion sequences (IS), Heffron with transposon 3 (Tn3), Kleckner with Tn10 and Toussaint and Resibois with the bacteriophage Mu, for which transposition is a way of life. Site-specific integration and excision is described by A. Campbell, using the example of bacteriophage lambda, for the elucidation of which he was himself largely responsible. Controlled segmental inversion as a means of reversible differentiation in bacterial and bacteriophages is represented by phase variation in *Salmonella typhimurium* in the chapter by Simon and Silverman. The *Salmonella* inversion-switch also appears in the context of Tn3, with which it appears to share a common mechanism and, presumably, a common origin. This example serves to emphasize the likelihood that "selfish" DNA

sequences will sometimes be "recruited" to provide useful functions in the regular genome.

Among the chapters dealing with eukaryotic systems, that by Varmus on retroviruses is of central importance. He stresses the extraordinary sequence similarities seen between parts of retrovirus genomes, several kinds of roaming *Drosophila* elements (reviewed by Rubin) and the Tyl element of yeast (described by Roeder and Fink). The suspicion grows that many of the dispersed, repetitive and occasionally movable sequences that bulk so largely in higher eukaryote genomes may be spread by retrovirus-like reverse transcription.

Bregliano and Kidwell provide an integrated account of the different agents of hybrid dysgenesis in *Drosophila melanogaster*. The molecular affinities of these elements have yet to be determined, but their properties show a striking resemblance in several respects to those of the maize transposable elements described in the 1950s by Barbara McClintock and reviewed here by Nina Fedoroff. There are further chapters (by Schell *et al.*, Haber and Borst respectively) on the *Agrobacterium*-Ti plasmid-plant system (essential

reading for would-be plant genetic engineers), and on the yeast mating-type and trypanosome surface glycoprotein switch mechanisms. The last two topics, like vertebrate immunoglobulin gene switching (not included here) perhaps really belong in a different book, but it is good to have them so well reviewed.

The editor, in his introduction, remarks that "these thoughtful reviews will not quickly become dated", and I agree with him. At the same time, they will quickly become supplemented by new information. Several of the systems described, notably the maize elements and the *Drosophila* hybrid dysgenesis elements, have only recently been cloned and are now being sequenced. In a few years' time we shall know much more, and it may be possible to propose a rational taxonomy of movable elements based on structure, transposition mechanism and presumed evolutionary relationships. This book is to be heartily welcomed, but we shall soon need another one. I hope that Dr Shapiro will undertake it. □

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Petal pursuits

Peter D. Moore

Double Flowers: A Scientific Study.

By Joan Reynolds and John Tampion.
Pembroke Press, 16 Pembroke Road,
London W11 3HZ: 1983. Pp. 183. £9.75.

SO WELL established is the historic divergence of horticulturists and academic botanists that many of the processes which the former take for granted are casually neglected by the latter. Among such is the phenomenon of double flowers. Even the term leaves much to be desired, for it includes a range of floral modifications such as petal proliferation, dissection and the adoption of petaloid form by other floral organs. The authors of *Double Flowers* have attempted to bring together the scattered fragments of information about this subject in the literature, which commences with the observations of Theophrastus in the third century BC. They also seek to classify double flowers on morphogenetic criteria and to examine their physiological and genetic basis.

One of the main causes of the average plant scientist's suspicion of things horticultural undoubtedly stems from the use in the latter of English variety names which provide little information concerning parentage. The authors here do nothing to ease such doubt in their use of English names even for wild plants in which doubleness has been observed. There is a pervading scent of imprecision here.

What could have been the most valuable

chapters in the book are those which review morphogenetic and physiological research in this area. The literature covered is very full and, undoubtedly, the bibliography will prove valuable, but the chapters suffer from the problem that the authors assume that the reader will already be aware of previous work. For example, "The work of X (1957) and Y (1975) is still, of course, interesting". This is hardly likely to inform the uninitiated.

Perhaps this style is explained by the fact that the book has developed out of a doctoral thesis. This becomes even more evident in the second part of the work, when five case studies of doubleness are considered. Four of these are reported in great detail and have clearly been the objects of the authors' own research. The wealth of practical detail and the tabulated results, together with the well-produced photomicrographs, contrast strongly with the earlier chapters.

Double Flowers has succeeded in two respects. It has set up a classificatory scheme for "doubleness" based in part upon the original floral structure (polypetalous, sympetalous, etc.) and in part upon the morphogenetic origin of the aberration. It has also provided some detailed analyses of a range of such floral types. What the authors have not succeeded in doing is presenting the subject adequately to those on the fringes (physiologists, morphologists, geneticists, taxonomists, etc.). But perhaps this is asking too much of a study which is evidently still in its infancy. □

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